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Animal by-products (ABPs): origins, uses, and European regulations

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Forewords

About half of each animal bred for meat, eggs and milk production, is not used for human consumption. A part of that unused half is subjected to rendering processes, resulting in many useful products. In the past industrial applications have used leather and skins, wools, fat and fatty acids as a feedstock, whereas relatively few applications were developed for animal by-products beyond adhesives and fertilizers. During the last decades the demand for recovering by-products for these applications has declined with the increase in use of widely available petroleum/synthetic-based products. A greater focus on renewable raw materials, however, has currently been reported, putting the focus on co-products otherwise destined for disposal.

In order to add value to animal hide and internal organs by-products, the meat industry is using science and research to add a value far beyond the usual profitability. It is necessary to employ up-to-date research tools to study these co-products for their properties, in order to develop new technological applications, and to search for medical, cosmetic or other industrial applications.

Leoci, in the present study, equipped with a large number of references (over 500), reviews the current uses of the various components of animal by-products--such as bile, blood, bones, brains, fats and fatty acids, glands (adrenal, kidney, liver, pancreas, etc.), hides, hearts, intestines, lungs, ovaries, stomachs, trachea, etc.--to show the "state of the art" of this science so that interested readers (operators of slaughter, transport and disposal of the unusable parts) and scholars may know in which direction we need to work and study in order to undertake further research for recovering additional useful components. The animal by-products are to be considered a mine of substances and compounds, still in large part undiscovered.

In the meantime, it should be noted, again warns Leoci, that the unusable parts wasted need to be handled with caution to avoid problems of environmental pollution and health hazards. Of course, the field of waste management is governed by numerous laws and regulations that must be observed to avoid problems and penalties. Leoci examines and lists the rules governing the management sector of the animal by-products, both that of waste (from the collection and transport to the recovery and/or disposal) and the overlapping and intersecting rules of animal by-product waste management in Europe and in Italy, highlighting the rules that cause problems.

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In this remarkable work Leoci, stepping outside her usual field of animal reproduction research, provides an overview of the state of the art of Animal By-Product (ABP) utilization. Animal slaughter currently generates a large amount of waste. Despite the progress over years past and recommendations of recent studies, approximately 50% of the weight of a slaughtered animal finds no use at all and is disposed of in landfills or, at most, used as a component in fertilizer. It's an unacceptable practice and a waste of renewable resources.

Among the various ABPs, greater attention needs to be paid to the rare recoveries and uses of animal's reproductive organs, which, in this paper, Leoci gives notice. These organs are rich in enzymes, hormones, vitamins, estrogens and countless other beneficial compounds that can address deficiencies and dysfunctions in both the animal and human kingdoms. However, further studies are required to hone accuracy and to find and define the appropriate application for the countless substances present in the animal reproductive organs. Studies and research is necessary not only to identify additional probable use in the pharmaceutical industry, but also and above all to better understand the complex mechanisms of cell reproduction, which until now is not completely clear. It is important to note that initial results in this area of cell reproduction were obtained by the study of sea urchin eggs.

This book will therefore be useful not only to scholars, providing a wide scope of bibliographical data (or notes) about relevant and recent research, but also to the workers and management of the slaughtering industry, possibly encouraging the industry to fund further studies in the field and thereby create an opportunity to reap economic returns thanks to the increased use of ABPs. It will also be useful to those who are concerned with European rules and regulations in the slaughtering industry and whose understanding of the issues will be deepened by the information.

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Preface

Animal by-products (ABPs) arise mainly during the slaughter of animals during the production of animal origin products (such as dairy, fibre, leather products), as the result of disease control measures, and in the course of the disposal of dead animals. Past and recent crises related to outbreaks of foot-and-mouth disease, the spread of transmissible spongiform encephalopathies (TSE) and the occurrence of hazardous chemicals (e.g. compounds of lead, mercury, chromium, PCBs, dioxins, etc.) in feeding stuffs have shown the potential risk to public and animal health and the environment. This risk needs to be adequately controlled, either by directing such products towards safe means of disposal or by using them for different purposes, provided that strict conditions are applied which minimize the health risks involved. On the other hand the disposal of all ABPs is not a realistic option, as it would lead to unsustainable costs for producers and consumers and risks for the environment. Conversely, there is a clear interest for all citizens that, provided the health risks are minimized, a wide range of ABPs can be safely used for various applications in a sustainable manner.

A lot of ABPs are commonly used in important productive sectors, such as in the pharmaceutical, feed, wool and leather industries but, notwithstanding, new technologies have widened the possible use of ABPs and derived products. Consequently a wide range of ABPs are not utilized and are destined to disposal.

This research first exposes the industry's "state of the art", namely what parts of ABPs are used to produce more food and useful products for various industries besides the traditional uses of fats and acids to produce energy or soaps; of hides and skins to produce footwear, articles of apparel, gelatines, and glues; and of bones to produce fertilizers, special ceramics, and glues. In addition, it shows there are many industries that use bile, blood, and glands to produce a long list of pharmaceuticals. It also shows that many other by-products components have yet to be studied in order to find suitable uses.

However, by eliminating all components used in various industries, including the food industry, there still remains a significant portion of ABPs that must be disposed. Part II mentions various possible disposal systems now in use: from sanitary landfills, to incineration with or without energy recovery, to anaerobic digestion in order to produce biogas.

In the European Union the whole sector is governed by rules and laws that can be divided into two branches: those governing the production, collection, transport and use of ABPs that are usable and those governing the collection, transport and disposal of ABPs that can not be used, since they are considered waste. In addition, Part II examines the various European rules (Regulations, Directives, etc.), adopted by all member nations and those specific to enforcement in Italy. It has been noted that some of the same ruling applications are not common to the two governing spheres. Some judgments of the Italian Constitutional Court, make it possible to avoid a few mistakes and related penalties required by the EU, but it would be appropriate to call on legislators to eliminate that uncertainty.

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PART I

Current uses of ABPs

1. Introduction

Until 1986 the use of meals of animal origin as a protein supplement in cattle had no restriction. In that year, the first cases[1] of Bovine Spongiform Encephalopathy (BSE) were identified in GB. Many studies suggested that the first cases occurred around April 1985.[2] BSE, commonly known as mad cow disease, is a fatal neurodegenerative disease (encephalopathy) in cattle[3] that causes a spongy degeneration in the brain and spinal cord. The disease has become known to the public as Mad Cow Disease (MCD). The BSE belongs to a group of diseases called "transmissible spongiform encephalopathies" (TSE), affecting different animal species, including humans.[4]

The occurrence of the disease, caused by a strange pathogenic protein known as "prion" or more commonly as an "unconventional infectious agent", was attributed to the use of animal meal as protein supplement in cattle, especially in the United Kingdom, where the rules, on the high temperature treatment of animal by-products, were much less restrictive than in other countries. The use of animal by-products (ABP)[5] of sick cattle, in the production of meat meal and bone meal for animal feed, brought on the onset of BSE in cattle. Maternal transmission was recently considered possible.[6]

The existence of prions was discovered by Stanley Prusiner,[7] 1997 Nobel Prize in Medicine, who added these agents to the list of well-known infectious substances including

1. Wells G. A. H., A. C. Scott, C. T. Johnson, R. F. Gunning, R. D. Hancock, M. Jeffrey, M. Dawson, R. Bradley (1987), "A novel progressive spongiform encephalopathy in cattle", *Veterinary Record*, vol. 121(18): 419-420.

2. Wilesmith J. V. (1991), "Epidemiology of bovine spongiform encephalopathy", *Seminars in Virology*, vol. 2: 239-245.

3. In the UK they are not immediately understood the link between the feeding of cattle with meat and bone meal and the rise of BSE. Refer for more news: Packer R. (2006), *The Politics of BSE*, Palgrave Macmillan, London; Wilesmith J. W., G. A. H. Wells, J. B. M. Ryan, D. Gavier-Widen, M. M. Simmons (1997), "A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy", *Veterinary Record*, vol. 141: 239-243.

4. See among others: Scoccia E., M. De Curtis, M. Biagetti, L. Faccenda, C. Maresca (2010), "Encefalopatia spongiforme bovina: excursus epidemiologico 2003-2008 - Bovine spongiform encephalopathy: epidemiological overview 2003-2008", *Sanità Pubblica Veterinaria*, vol. 63 (12). (http://indice.spvet.it#500).

5. "Animal by-Products (ABPs)" can be defined as animal carcasses, parts of carcasses or products of animal origin (including ova, embryos and semen, sometimes glands, bloods, etc.) that are not intended for human consumption.

6. Wilesmith J. W., G. A. H. Wells, J. B. M. Ryan, D. Gavier-Widen, M. M. Simmons (1997), *Veterinary Record*, ibid.: 239-243.

7. S. B. Prusiner was Professor of Neurology at the University of California, San Francisco where he has worked since 1972. From 1969-72, he served in the US Public Health Service at the National Institutes of Health. He is the recipient of numerous prizes, including the Nobel Prize in Physiology or Medicine (1997). Prusiner's work helped the world to understand more about Alzheimer's and Mad Cow disease through his discovery of the prion, a disease-causing agent like bacteria or viruses (See: Prusiner S. B. (1991), "Molecular Biology of Prion Desease", *Science – New Series 1991*, vol. 252 (5012): 1515-1522). Prions cause transmissible and genetic neurodegenerative diseases, including scrapie and bovine spongiform encephalopathy of animals and Creutzfeldt-Jakob and Gerstmann-Sträussler-Scheinker diseases of humans. The prion protein can manifest itself as two proteins, one an innocent "Dr. Jekyll" character, while the other, dangerous "Mr Hyde" protein causes disease and death.

bacteria, viruses, fungi and parasites. Prions exist normally as innocuous cellular proteins; however, they possess an innate capacity to convert their structures into highly stabile conformations that ultimately result in the formation of harmful particles, the causative agents of several deadly brain diseases of the dementia type in humans and animals. Prion diseases may be inherited, laterally transmitted, or occur spontaneously.

While a neurology resident, Prusiner was in charge of a patient who died of a rare fatal degenerative disorder of the brain called "Creutzfeldt-Jakob Disease"[8] (vCJD o nvCJD). Prusiner became intrigued by this little-known class of neurodegenerative disorders, the spongiform encephalopathies that caused progressive dementia and death in humans and animals. In 1974 he set up a laboratory to study scrapie, a related disorder of sheep, and in 1982 he claimed to have isolated the scrapie-causing agent. He claimed that this pathogenic agent, which he named "prion," was unlike any other known pathogen, such as a virus or bacterium, because it consisted only of protein and lacked the genetic material contained within all life-forms that is necessary for replication.

When first published, the prion theory met with much criticism, but it became widely accepted by the mid-1990s. In 1996, when a new variant of "Creutzfeldt-Jakob Disease" emerged in Great Britain, Prusiner's research was the focus of national attention. Fears abounded that the new variant of the disease might be linked to "mad cow" disease, a brain disorder that first appeared in British cattle a decade earlier. Some evidence suggested that the mad cow prion might have jumped species, infecting humans who consumed beef contaminated with the infectious agent. Because mad cow disease was believed to have been caused when the agent that causes scrapie in sheep was transmitted to cattle in feed, there was precedent for species-jumping events to occur. Prusiner's research also could have significant implications for such disorders as Alzheimer disease and Parkinson disease, which seemed to share certain characteristics with the diseases caused by prions.

The leading scientific theory at this time maintained that CJD and the other TSEs were caused by a type of prion.[9]

8. Creutzfeldt-Jakob disease (CJD) is a rare, degenerative, invariably fatal brain disorder. It affects about one person in every one million people per year worldwide. CJD belongs to a family of human and animal diseases known as the transmissible spongiform encephalopathies. Spongiform refers to the characteristic appearance of infected brains, which become filled with holes. The causes and origins of Creutzfeldt-Jakob disease are not yet known. Some researchers believe an unusual "slow virus" or another organism causes CJD. However, they have never been able to isolate a virus or other organism in people with the disease. Furthermore, the agent that causes CJD has several characteristics that are unusual for known organisms such as viruses and bacteria. It is difficult to kill: "it survives" or better, it stays active after 30 minutes of immersion in boiling water, it is resistant to UV, to concentrated formaldehyde, to phenol. "Survives" because maybe it is not a living organism, but a protein "containing" DNA. It does not appear to contain any genetic information in the form of nucleic acids (DNA or RNA), and it usually has a long incubation period before symptoms appear. In some cases, the incubation period may be as long as 50 years.

9. Intriguingly, the infectious prion protein is made by the host, and its amino acid sequence is identical to a normal host protein. Moreover, the prion and normal forms of the protein are indistinguishable in their posttranslational modifications. The only difference between them appears to be in their folded three-dimensional structure. The misfolded prion protein tends to aggregate, and it has the remarkable capacity to cause the normal protein to adopt its misfolded prion conformation and thereby to become infectious. This ability of the prion to convert the normal host protein to misfolded prion protein is equivalent to the prion's having replicated itself in the host. If eaten by another susceptible

Kuru is a human prion disease, very similar to BSE that was spread from one person to another by ritual mortuary practices in New Guinea. However, the two Nobel Laureates, Gajdusek[10] and Prusiner, never found an explanation of the unusual features of prion. Additionally, biologist and Nobel Laureate James Watson[11] called prion the strangest thing in molecular biology. To date, its chemical structure remains unknown and no one is certain of having ever seen prion under a microscope.

2. Animal by-products (ABPs): what are they?

Slaughtered bred animals are commonly divided in two typical fractions: animal products used for food and not destined for food (edible and inedible ABPs). The amount of by-products not destined for food is what slaughterhouses refer to as their "5th quarter".

Proportion of meat and by-products (estimates)		
Slaughtered animal	**For human consumption (~%)**	**ABPs (~%) (edible and inedible)**
Cattle	55	45
Broilers	70	30
Pig	60	40
Sheep/goat	50	50

The above proportions are not valid for all countries. For instance, in India cattle are 100% inedible, while in Muslim countries pigs are 100% inedible. Even the proportions may vary according to many factors (breeds, breeding conditions, feeding, etc.). Regarding the ABPs, some parts (for instance livers, blood, lungs, etc.) are edible in some countries and inedible in other countries, depending on eating habits. Anyway, inedible by-products include all slaughter by-products which are obviously not fit for human consumption: leathers, feathers, bristles and horns or materials that have been declared not fit for human consumption after a veterinary inspection (infected meats, injured animals, meat with inflammation or bad smells, etc.). Inedible by-products also include all animals that happened to die on farms or were killed for animal disease eradication, and animals that are not used for human consumption (zoo and circus animals, pets and fur animals, animals used for experiments and studies, etc.).

host, these newly-misfolded prions can transmit the infection. It is not known how normal proteins are usually able to find the single, correct, folded conformation, among the billions of other possibilities, without becoming stuck in dead-end intermediates.

10. Daniel Carleton Gajdusek was a Hungarian-Slovak-American medical researcher who was the co-recipient (with Baruch S. Blumberg) of the Nobel Prize. Gajdusek had the rare distinction of being a Nobel prizewinner (in Medicine in 1976 for work on kuru) and a convicted child molester. Kuru is an incurable disease that affects the Fore tribe in Papua New Guinea. Gajdusek showed that it had a long incubation period, but progresses rapidly when it starts.

11. James Dewey Watson was a biologist, U.S., discovered the structure of the molecule of DNA with Francis Crick and Maurice Wilkins, with whom he also received the Nobel Prize for Medicine in 1962.

In theory, about 95% of one animal is usable. The remaining 5% are processing losses. From that 95%, about 55% (on average) of the animal is used for edible products and the remaining 45% are inedible by-products. According to Meeker and Hamilton[12] approximately 49% of the live weight of cattle, 44% of the live weight of pigs, 37% of the live weight of broilers, and 57% of the live weight of most fish species are materials not consumed by humans. A part of these last by-products are, at times, transformed into a variety of products used in human food, animal feed, cosmetics, pharmaceuticals and other technical uses.

Edible animal by-products have traditionally been used as a source of digestible protein, nutrients, and energy in the feed and animal food industries.

The world production of ABPs derived from the meat and animal production industries is approximately 60 million tons per year. It has been estimated that more than 10 million tons of products not destined for direct human consumption, derived from healthy animals, are produced in the EU every year. During slaughter, between 33 and 43% by weight of the live animal is removed and discarded as useless waste. A part of these materials, mostly offals[13] are collected and processed by the rendering industry to produce raw materials that are used in the animal feed industries around the world.[14]

Offal, not used directly for human or animal food,[15] is often processed in a rendering plant producing material that is used for fertilizer[16] or fuel. In some cases, it may be added to commercially produced pet food.

Typically wastes derive during the processing of ABP and animal products, and more generally can be identified as occurring from food processing and manufacturing plants, distribution premises, food markets, wholesale and retail food outlets, and catering facilities (including household kitchens). On some or any of these sites animal product processing derivatives include parts of animals deemed unfit for human consumption: butcher and slaughterhouse waste, catering waste, wool, blood, feathers, hides and skins, used cooking oil, eggs, former foodstuffs, fallen stock,[17] manure, embryos and semen. The by-products

12. Meeker D. L., C. R. Hamilton (2006), *An Overview of the Rendering Industry*, in Meeker David L. (Edt), *Essential Rendering. All About The Animal By-Products Industry*, National Renderers Association, Printed in September of 2006 by Kirby Lithographic Company, Inc., Arlington, Virginia (USA), p. 2.

13. Offals are the entrails and internal organs (blood, tongue, throat, stomach, brains, honeycomb tripe, tripe, trepas, tails, sweetbreads, lips, kidney, liver, etc.) of a butchered animal. The word does not refer to a particular list of organs, but includes most internal organs which are then used in human food, animal feed, cosmetic, pharmaceutical and technical products.

14. Hamilton C. R. (2002), *Real and perceived issues involving animal proteins, Protein Sources For The Animal Feed Industry*, Expert Consultation and Workshop Bangkok, FAO: 29 April - 3 May, 2002.

15. HeartSome offal dishes are considered gourmet food in international cuisine. This includes French foie gras and pâté and sweetbreads. Other offal dishes remain part of traditional regional. This includes Scottish haggis (sheep stomach stuffed with a boiled mix of liver, heart, lungs, rolled oats and other ingredients), USA chitterlings (intestines), Jewish chopped liver as well as many other dishes. Intestines are used as casing for meat sausages or blood sausage (boudin noir, or the Spanish "morcilla", or Italian "sanguinaccio") For further notices see: Helou A. (2004), *Offal, the Fifth Quarter*, Absolute Press, Scarborough House, Bath, UK (last edition 2011).

16. Sharrock P., M. Fiallo, A. Nzihou, M. Chkir (2009), "Hazardous animal waste carcasses transformation into slow release fertilizers", *Journal of Hazardous Materials*, vol. 167 (1-3): 119-123.

17. "Fallen stock" can be any animal that has died of natural causes or disease on the farm or has been killed on the farm for reasons other than human consumption.

are destined for destruction because they are not usable in the consumer market or to the fertilizer or the livestock industries, nor are they suitable for the production of biogas or for composting. In other words, if no part of the ABPs is separated and used, the ABPs, on the whole, are considered waste that must be disposed.

ABPs and wastes are a potential source of risks to public and animal health. Improper use of ABP has resulted in outbreaks of serious diseases such as bovine spongiform encephalopathy, and as already said, foot and mouth disease, avian influenza, classical swine fever, etc.

3. Historical uses of ABPs

The process of rendering animal parts has been documented for more 2,000 years.[18] The purpose of rendering was to produce tallow and other animal fats to make candles and soap.

In the past, non-food, non-feed applications for rendered ABPs, with the exception of tallow, were limited in their application to niche markets[19] that were too small to support large volumes of meat and bone meal.

Nowadays, while muscles are the more consumed part of the animals, ABPs such as the entrails and internal organs are also widely consumed. The use and value of inedible meat by-products and offal-edible, considered waste material to be thrown away or high-priced delicacies, depend on the country in question and its culture. The blood, bones, meat trimmings, skin, fatty tissues, glands, horns, hoofs, lags and feet, and skulls of harvested animals, comprise a wide variety of products. When slaughtering healthy animals and collecting slaughterhouse debris, those natural and environmentally compatible by-products become raw materials for the industry in general.[20]

Historically, non-feed, non-food applications for ABPs, excluding tallow, has tended to be limited in their application to niche markets.[21] Historically, "rendering" has been defined as separation of fat from animal tissues by the application of heat. Rendering can refer to any processing of animal products into more useful materials, or more narrowly to the rendering of whole animal fatty tissue into purified fats like tallow or lard. Rendering can be carried out on an industrial, farm, or kitchen scale.

Meeker and Hamilton[22] noted that "one-third to one-half of each animal produced for meat, milk, eggs, and fiber is not consumed by humans. These raw materials are subjected to rendering processes resulting in multiple useful products. Meat and bone meal, meat meal, poultry meal, hydrolyzed feather meal, blood meal, fish meal, and animal fats are the primary products resulting from the rendering process". The same authors stated, "A by-product is defined as a secondary product obtained during the manufacture of a principal commodity. A co-product is a product that is usually manufactured together or sequentially

18. Grummer R. R. (1992), *Inedible Fats and Greases*, Chapter 6, Edts., A. E. Pearson and T. R. Dutson, *Inedible Meat By-Products*, Elsevier Applied Science, London-New York, pp. 113-148.
19. Pearl G. G. (2003), Non-Food/Feed Uses of Rendering Products: Identification of New Opportunities and Assessment of Major Barriers to their Exploitation, Fats and Protein Research Foundation, Research Project ♯01B-6, Bloomington, Illinois (USA), pp. 4-35.
20. Ockerman H., C. L. Hansen (2000), *Animal by-product processing & utilization*, Technomic Publishing Company Inc., Lancaster (PA- USA).
21. Pearl G. G. (2003), "Non-feed, non-food applications for animal by-products", *Render*, 32 (1): 22-25.
22. Meeker D. L., C. R. Hamilton, *An Overview of the Rendering Industry*, ibid., p. 1.

with another item because of product or process similarities". We will preferably use the term by-product. Rendering is a process of both physical and chemical transformation using a variety of equipment and processes. All of the rendering processes involve the application of heat, the extraction of moisture, and the separation of fat.

According to Romans and others,[23] rendering involves the heating or cooking of raw materials, with complex or simple mixtures of protein, minerals, and fatty substances, to liquefy fats and break down membranes or other structures that may hold fat. According to Kumar,[24] the goals of carcass rendering are elimination of water, separation of fat from other materials (mainly protein substances), sterilization of the final products, and production of MBM[25] from a variety of condemned, fallen, culled, and experimental animals.

Prokop,[26] UKDEFRA,[27] Romans and others defined rendering as a process of using high temperature and pressure to convert whole animal and poultry carcasses or their by-products with no or very low value to safe, nutritional, and economically valuable products. In fact, the highly perishable protein and fat materials comprising carcasses become a major problem and a liability if they are not converted, stabilized, or somehow processed during the 24 hours following death.

A lot of industrial chemical applications have used fat and fatty acids as a feedstock, whereas relatively few applications were developed for ABPs beyond adhesives and fertilizers. The outbreak of World War I and II saw great demand for glycerin used in the production of explosives (tri-nitroglycerin). The demand for recovering co-products for these applications has declined with the increase in use of widely available petroleum/synthetic-based products.

In order to add value to animal by-products of the hide and internal organs, the meat industry is using science and innovation.[28] Industry is using science and research to add value to ABPs far beyond its usual profitability. It is still necessary to employ the most up-to-date and effective research tools to study these products for their nutritional properties, to search for medical, cosmetic and in other industrial fields, to develop new technological applications and to continue innovation towards advanced value-addition of ABPs.

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23. Romans J. R., W. J. Costello, C.W. Carlson, M. L. Greaser, K. W. Jones (2001), *Packing house by-products*, in *The Meat We Eat*, Interstate Publishers, Inc., Danville, Illinois; Jiménez-Colmenero F., M. Reig, F. Toldrá (2006), *New Approaches for the Development of Functional Meat Products*, Chapter 11, in L. M . L . Nollet and F. Toldrá (Edtrs), *Advanced Technologies For Meat Processing*, CRC Press, Florida (USA), pp. 275–308.

24. Kumar M. (1989), *Handbook of rural technology for the processing of animal by-products* (FAO Agricultural Services Bulletin No. 79), FAO of the United Nations, Rome.

25. MBM (Meat and Bone Meal) is prepared from the rendering of dead animals or wastes materials associated with slaughtering operations (carcass trimmings, condemned carcasses, condemned livers, inedible offal and bones). It is basically dry rendered protein product from mammal tissues with more than 4.4% phosphorus.

26. Prokop W. H. (1996), *The rendering industry – a commitment to public service*, in D.A. Franco & W. Swanson (Edts.), *The original recyclers*, Joint publishers: the Animal Protein Producers Industry, the Fats & Proteins Research Foundation, and the National Renderers Association, Merrifield, VA (USA).

27. UKDEFRA (2000), *The BSE inquiry report*, Vol. 13: *Industry processes and controls*, Ch. 6 rendering, Annex B: manufacturing processes of rendering.
(See: http://www.bseinquiry.gov.uk/report/volume13/chapterj.htm).

28. See: Toldrá F., M. Concepción Aristoy, M. Reig (2012), "Innovations in value-addition of edible meat by-products", *Meat Science*, 92 (3): 290-296.

As noted by Peter Kent in Clemson Impacts:[29] "The new Clemson Animal Co-Products Research and Education Centre was dedicated in March (2006). This research initiative will serve the rendering industry, which collects and processes billions of pounds of animal remains, the inedible leftovers from meat production. Clemson researchers will seek new ways to recycle fats and protein from food animal production to create a variety of value-added products, including bio-fuels, fertilizers, soap, rubber, and plastics. Also, the center will work to ensure the safety of rendered products for animal feeds and consumer products, promote environmentally sound practices, and provide educational opportunities in utilizing animal co-products".

McGlashan,[30] in his preamble for the Clemson University Animal Co-Products Research and Education Center dedication conference (April 2006), states: "It is imperative to society that the rendering industry remains viable". As stated in the book's first chapter, "Overview of the Rendering Industry", the availability of rendered products for animal feeds in the future depends on regulation and the market. Future regulation relating to biosecurity and environmental protection has the potential to restrict traditional market access for rendered co-products. Hence, it is essential that new applications and avenues for profitable disposal of co-products are discovered, researched, developed into a viable commercial process, and widely adopted by the industry in order to maintain rendering as a viable and valuable service to the meat processing sector".

Researchers at many universities believe that these inedible parts of carcass may have value. Researchers launched their research initiative after finding that there is a lack of central location for expertise in determining value from animal agriculture that is not simply muscle related. For example, Schaefer[31] stated: The mission "is really to discover new uses of known bioactive molecules, mass produced from by-products of animal and poultry that have been produced for meat, and to discover novel compounds that can be used in biology, agriculture, medicine and other consumer products".

The UKDEFRA[27] recognized that animal waste collection and rendering "constituted a vital public service as well as commercial activity" but made some recommendations intended to remedy the effect on competition of these firms' pricing policies.

4. Current uses of ABPs

At present, the main ABPs useful for foods, industrial products and pharmaceutical uses, are the following:

- Bile: detergent, pharmaceuticals;
- Blood:
  - ***a.*** Edible blood: additives, binders or meat glue, blood flours, blood proteins, blood sausages, cake mixes, clarifiers, coffee whiteners, curing agents, deep-fry batters, dyes and color enhancers, egg albumin substitutes, emulsifiers, fat replacer, gravy mixes, imitation seafood, pasta, stabilizers, whipped toppings;

29. Clemson Impacts, a quarterly publication of Clemson Public Service Activities, is available on the web www.clemson.edu/public/.

30. McGlashan S. A. (2006), *Industrial And Energy Uses Of Animal By-Products, Past And Future*, conference held at the Madren Conference Center on campus in March 27-29, 2006, p. 242.

31. D. M. Schaefer, Professor and Department Chair, Department of Animal Science, 256A Animal Science Bldg, 1675 Observatory Drive, Madison, WI 53706.

b. Feed (blood meal): lysine supplement, milk substitute, nutritional component, and vitamin stabilizer;

c. Fertilizer: mineral components, seed coating, soil pH stabilizer;

d. Scientific use or research: albumin, antibodies, antiserum, blood clotting factors, leukocytes - white blood cells, plasma, red blood cells, serum, whole blood, antigens;

e. Laboratory uses: serum, culture media, tannin analysis, active carbon, haemin, blood agar, fibrinogen, peptone, glycerophosphates, coagulation factors, blood albumin, RH factor typing, globulins, sphingomyelins, catalase, agglutination tests, diagnostic microbiology;

f. Pharmaceutical use: immunoglobulin, thrombin (blood clotting), factors, fibrinogen, fibrinolysin, fibrin products, serotonin, kallikreins, plasminogen, plasma extenders, transfusion. Products usually for manufacturing cosmetics, human nutritional supplements, and test kit components;

g. Veterinary biological use (also called veterinary biologics);

h. Industrial uses: glue and resin extender, finishes for leather and textiles, insecticide spray adjuvants, egg albumin substitute, foam fire extinguisher, porous concrete, ceramic and plastic manufacture, plastic and cosmetic base formulations.

- Bones: adhesives, animal feed, bandage strips, bone meal (calcium and phosphorous source), bone marrow (blood disorders), cold cream, collagen and bone for plastic surgery, crochet needles, cutlery handlers, dice, dog biscuits, emery boards and cloth, glycerin, fertilizer, glue, gelatin, hairpins, imitation ivory, inedible bone meal, livestock feeds, meal, mineral source in supplements, neat fat oil, piano keys, photographic film, plant food, plywood and paneling, protein hydrolysate, syringes, soft cartilage (plastic surgery), shampoo and conditioner, tallow and ornaments, wallpaper and wallpaper paste, xiphisternal cartilage (breastbone);
- Brains and spinal cords: steroid, cholesterol, lecithin, cephalin;
- Fats and fatty acids: antifreeze, animal foods, biodegradable detergents, biodiesel, cellophane, cement, ceramics, chalk, chemicals, cosmetics, crayons, creams and lotions (sheep), deodorants, detergents, explosives, fertilizer, fiber softeners, floor wax, glycerin, glycerol, herbicides, horse and livestock feeds, industrial oils, insecticides, insulation, linoleum, livestock feed, lubricants, lubricating greases, makeup, matches, medicines, mink oil, nitroglycerine, oil polishes, ointment bases, oleostearin, paints, paraffin, perfumes, pet foods, pharmaceuticals, plasticizers, plastics, printing rollers, protein hair conditioner, protein hair shampoo, putty, rubber products, shaving cream, shoe cream, soaps, solvents, stearic acid (sheep), tallow for tanning, textiles, tires, water proofing agents, weed killers;
- Glands:

a. Adrenal: cortisone (for arthritis, skin allergies, anti-inflammatory medicine), epinephrine (aid in raising blood pressure, heart disorders, and allergies);

b. Kidney: pharmaceuticals and others (for transplantation);

c. Liver: liver tonics, heparin (anti-coagulant, prevents gangrene), liver extract (treat-

ment of anemia), intrinsic factor (pernicious anemia), Vitamin B12 (prevention of B-complex deficiencies);

d. Pancreas: chymotrypsin (contact surgery), diastase (aid in starch digestion), glucagon (treat hypoglycemia), insulin (diabetes mellitus), pancreatin (aid digestion), trypsin (for burns, wounds, and infection, promotes healing, aid in protein digestion and in cleaning wounds);

e. Pituitary glands: ACTH (arthritis, allergies, rheumatic fever, skin and eye inflammations), pressor hormone (regulates blood pressure), prolactin (promotes lactation), vasopressin (controls intestinal and renal functions);

f. Spleen: in addition to food, ferritin, for stimulating the immune system;

g. Thymus: thymosin, thymopoeitin, thymulin, thymic humoral factor, thymus extract, as food;

h. Thyroid (Thyrar): TSH (thyroid diagnosis thyroid extract - hypothyroidism, thyroid hormones, myxedema cretinism.

- Hides and skins: bandages, belts, bookbinding, cabinetmaking, collagen-based adhesives (from trimmings), drum head (sheep), emery boards, gelatin, gloves, and shoes, glues-for papermaking, leather, leather sporting goods, leather wearing apparel, luggage, pharmaceuticals, photographic materials, pigskin garments, porcine burn dressings for burn victims, sheetrock, shoes and boots, upholstery, wallets, wallpaper;
- Hairs and wools, skins, feathers, nails, horns, and hooves: air filters, artist's paint brush, brushes, fabrics, feather meal, crochet needles, hairpins, imitation ivory, horn handles, cutlery handlers, buttons, cellophane wrap and tape, laminated wood products, chessmen, combs, protein hydrolysate, fibers, felt and rug padding, insulation material, non-woven, plastering material, textiles, upholstering material, yarn;
- Hearts (hog heart valves for human transplant);
- Intestines: sausage casing, strings (for suturing material, surgical ligatures, musical instruments, racquets), heparin. Small Intestine Submucosa (SIS materials);
- Lungs: heparin (anti-coagulant, prevents gangrene), meal;
- Meat and bone meals (pyrolysis, fluidized bed reactor, anaerobic digestion, co-firing/incineration, concrete and asphalt construction);
- Ovaries: estrogen (progesterone, relaxin, etc.);
- Proteins: protein hydrolysis, plastics, adhesives, surfactant;
- Stomach and tripe: pepsin (aid in protein digestion), rennet (aid in milk digestion);
- Trachea: chondroitin sulphate.

As can be seen, some products can be obtained from various components of the ABPs, for example glues and gelatins. Blood, bones, skins, horns, feathers, as we will see later, can be used for production of glues, while bones, skin and connective tissue are used for the production of gelatin which is then used in human food, animal feed (coating on vitamins, and binders on feed pellet and dog chews), pharmaceutical manufacturing (hard and soft capsules) and technical use (in the photographic industry, for example, as paper coating and as a component in silver halide emulsion coatings, etc.). Gelatin is the main ingredient in

products like Jell-O and Gummy Bears and can be found in many other product, such as yogurt. Gelatin is usually made by boiling the skin, tendons, ligaments, and bones of cows and pigs after the meat has been harvested. It can also be made from fish or other animals. The alternative to gelatin is "agar-agar"[32] which is produced from seaweed.

Fertilizer and soil conditioners are a minor factor for ABPs while there is widespread and profitable use of protein-based meals in the feed and food sector and valuable use of many components of ABPs in different industrial sectors.

The products for general food are the following:

1. from cattle, sheep, hog flesh: a great variety of fresh, frozen, and pre-cooked meats and prepared and processed meat products;
2. from milk/dairy: butter, casein, cheese and cheese products, cream, food ethanol, ice cream and ice cream mixes, lactose (carbohydrates), milk powder, sherbet, whey (proteins), fats (lipids), yogurt;
3. from fats and fatty acids: chewing gum, lard, oleo margarine, oleo shortening, oleostearin, pharmaceuticals, rennet for cheese (sheep), rennet for cheese (sheep), shortening.

Some of the above by-products contain highly valued compounds. The high market price of these compounds/products provide profitable opportunity for the "mining" of lipoproteins, peptides, enzymes, hormones, insulin, etc. that can be extracted from glands and organs, concentrated, dried for storage, and further processed or sold as raw material for other industries.

The likely future use of organs (hearts, livers, intestines, lungs, etc.) taken from genetically modified animals may become objects of primary production, while the meat from these animals could assume the role of ABP, if not intended for human consumption.

Premises handling ABPs include:

- rendering plants;
- handling and storage plants (previously known as intermediate plants);
- incinerators/co-incineration plants;
- composting and anaerobic digestion (biogas) plants;
- pet food plants;
- technical plants;
- collection and treatment plants for ABP;
- final users of treated ABP;
- pet food plants;
- rendering plant.

Let us take a closer look at the most important uses of above mentioned ABPs.

32. Agar-agar is a polysaccharide that comes from Japan used as a gelling natural. It derives from red algae belonging to different genera (Gelidium, Gracilaria, etc.). It is a polymer consisting of units of D-galactose. The galactose is one of the two components of the lactose, that is a disaccharide formed from one molecule of α-glucose and of a β-galactose.

4.1 Bile

Bile is a digestive fluid that is made and released by the liver and stored in the gallbladder. Bile helps break down fats into fatty acids, which can be taken into the body by the digestive tract. Bile contains mostly cholesterol, bile acids (also called bile salts), and bilirubin (a breakdown product of red blood cells). It also contains water and body salts (potassium and sodium), as well as very small amounts of copper and other metals.

The first studies on the use of bile salts as detergents (anionic detergents) date back to the years prior to World War II.[33] It was observed at the time, but the characteristic was already known,[34] as "the synthetic detergents and the bile salts all have the same general type of hydrophobic-hydrophilic structure. Each consists of a large hydrophobic part with a small hydrophilic part attached to it". Bile powder is used as nutraceutical for the treatment of indigestion, for constipation and for bile tract disorders. It is also used to increase the secretory activity of the liver and in aquaculture as a prawn feed ingredient. Mixed bile acids are also used in poultry and pig foods. Natural taurine[35] is used in infant milk formula to make it more like human milk, in health tonics ("smart drinks"), and for the prevention of side effects from excess alcohol consumption.

Gallstones[36] are reported to have aphrodisiac properties and can be sold at a high price. They are usually used as ornaments to make necklaces and pendants.

As pharmaceutical use, bile acids or their salts and conjugates[37] are formulated for nonsteroidal anti-inflammatory drugs, such as indomethacin,[38] and other pharmaceutically active compounds (antigen-vaccine preparation, cell culture, core bioreagents, etc.). Bile is manufactured from raw ox bile and contains a mixture of conjugated bile acids, namely a mix of bile salts, predominantly sodium glycocholate hydrate ($C_{26}H_{42}NO_6Na{\cdot}nH_2O$) and sodium taurocholate hydrate ($C_{26}H_{44}NNaO_7S{\cdot}nH_2O$) along with a small amount of free bile acids (cholic acid) and lipids. The bile salts are used as an ingredient in the manufacturing of dehydrated culture media, such as McConkey, VRBL, Electrophoresis Chromatography, and others. A major use is in clinical microbiology to selectively grow fecal staphylococci and streptococci (MacConkey Agar).

33. Anson M. L. (1939), "The denaturation of proteins by synthetic detergents and bile salts", *The Journal of General Physiology*, 22: 239-246.
34. See: Kühne W., L. Hermann (1879), *Handbuch der Physiologie*, F.C.W. Vogel, Leipsic, p. 264.
35. Taurine ($C_2H_7NO_3S$) is present in most animal tissues and is particularly abundant in oysters, mussels, bovine bile and human breast milk. It is one of the amino acids classified as non-essential, which means it can be made by the human body. Research also suggests that it might also protect cell membranes from toxic substances. In fish it is thought to be an osmoregulator of cell volume.
36. Gallstones are hard, pebble-like deposits that form inside the gallbladder.
37. See: Barnwell S. G., Pharmaceutical composition containing a low detergent effect bile salt and an active compound that undergoes biliary excretion and/or enterohepatic recycling, US Patent 5,942,248, 1999.
38. Indomethacin is a nonsteroidal anti-inflammatory drug that reduces fever, pain and inflammation. It is similar to ibuprofen and naproxen.

Ursodeoxycholic acid[39] is found naturally in bear bile[40]. It is an Asian folk medicine used to protect the liver. Modern medical research has proved that ursodeoxycholic acid is an effective drug in the treatment of liver disease.[41] Cholic acid ($C_{24}H_{40}O_5$)[42] is used as the starting material in the synthesis of ursodeoxycholic acid.

Cholic and deoxycholic acids are the starting point for the synthesis of many steroids. They are used as starting materials for other steroidal compounds such as gallstone-dissolving drugs, hepatic drugs, digestive aids, and corticosteroids.

Bile powder is used as nutraceutical to aid digestion and in aquaculture as a prawn feed ingredient. Mixed bile acids are also used in prawn feed and in poultry and pig foods also.

Natural taurine (an amino acid, not a steroid) is used in infant milk formula to make it more like human milk, in health tonics ("smart drinks"), and for the prevention of side effects from excess alcohol consumption.

It is used some ingredients of bile, such as prednisone and cortisone, can be extracted separately, and used as medicines.

4.2 Blood

Blood or blood fractions are derived from ruminants, birds or poultry, equine (horses), and swine.

Blood, as it is known, is composed of blood cells (40% of total blood volume) suspended in a liquid blood plasma. Its composition is 18-19% protein (fibrinogen) and 78-79% moisture. Blood cells are composed of red cell (erythrocyte), white cell (leukocytes), and platelets (blood clotting). Plasma contains globulins, albumins, fibrinogen, dissipated proteins, glucose, mineral ions, hormones, carbon dioxide, and blood cells themselves.

The color of beef plasma is yellow or orange and that of pig plasma is gray-white to pink. Blood plasma proteins, such as albumin and globulins, are good emulsifiers.

Animal blood amounts to 5-7% of the body weight of mammals (dried blood makes up to 0.7% of live weight). While half of the blood volume of a slaughtered animal remains in the carcass tissues (50% retained in the capillary systems throughout the body) and is eaten with the meat and internal organs, the other half recovered from bleeding represents 5-8 percent of the protein yield of a slaughtered animal. The remainder (42-45%), if it is not recovered may cause environmental pollution hazard. In the future, we cannot afford to waste such large amounts of animal protein.

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39. Ursodeoxycholic acid is one of the secondary bile acids, which are metabolic by-products of intestinal bacteria. The liver produces primary bile acids and stores it in the gall bladder. Primary bile acids when secreted into the intestine can be metabolized into secondary bile acids by intestinal bacteria. Primary and secondary bile acids help the body digest fats. Ursodeoxycholic acid helps regulate cholesterol by reducing the rate at which the intestine absorbs cholesterol molecules while breaking up micelles containing cholesterol. Because of this property, ursodeoxycholic acid is used to treat (cholesterol) gallstones non-surgically.

40. Bears suffer terribly when people extract the bile for medicinal use. Bears cannot be used as a source where large quantities of it are required as a pharmaceutical.

41. Paumgartner G., U. Beuers (2002), "Ursodeoxycholic Acid in Cholestatic Liver Disease: Mechanisms of Action and Therapeutic Use Revisited", *Hepatology*, 36 (3): 525-31.

42. One of the primary bile acids, usually found conjugated with glycine or taurine. It facilitates fat absorption and cholesterol excretion.

Blood used for transfusions is always human in origin, though some blood substitutes are made from animal sources. Many diagnostic laboratory tests use animal or human sourced reagents. In recent years transfusion medicine has become very prevalent in veterinary care. Animal blood banks do supply blood products.

The procedures for edible blood collection undergo different phases. Healthy animals must be inspected and passed for use as meat food. After collection, blood needs to pass inspection. Then it can be used for human utilization since blood taken from healthy animals is essentially sterile and any contamination is due to the bleeding technique and the drainage system employed during collection.[43] Many blood products are made from the plasma component of blood.

Let's take a look at the main uses.

a) Edible blood (Food)

The meat industries use the blood plasma and isolated blood proteins as ingredients in the food industry, mainly as a binder but also as natural color enhancers, emulsifiers, fat replacers, meat curing agents, etc. Blood plasma and blood proteins function as a binder in meat systems thanks to their ability to form gels upon heating. Other industries use blood albumin, meat glue and the bulk of the blood in blood sausage, soup, and as protein supplement.[44]

Additives

Blood is an abundant source of iron and proteins of high nutritional and functional quality. We will see below the use of proteins for alimentary use. Extensive studies and patent searches, on the functional properties of blood, have shown that blood plasma exhibits many functional properties, including, gelling, water holding, solubility, emulsification and foaming capacity.

Plasma is widely used in the food industry because it possesses a neutral taste and is devoid of the dark color associated with red blood cells. Instead, hemoglobin is utilized for its color. The uses are as follows:

- Porcine or bovine plasma, with increased fibrinogen concentration, is used as cold set binder for meat products;
- Bovine spray-dried plasma concentrate as emulsifier, gelling and binding agent in meat and fish-based products, pasta and bakery products;
- Porcine or bovine stabilized hemoglobin, porcine or bovine frozen or powder hemoglobin as natural coloring for meat products;
- Porcine or bovine natural colorant obtained from the red pigments of blood in order to enhance meat color and increase contrast between fat and meat;
- Bovine globin (hemoglobin with the heme group removed) as emulsifier in meat products;
- Porcine or bovine heme iron polypeptide for iron supplementation.

Blood and its other derivatives, has the potential to serve as a useful source of valuable peptides with antioxidant, anti-hypertensive, and anti-bacterial properties. Several bioactive

43. Riaz M. N. (2010), "Fundamentals of halal foods and certification", *Prepared foods*, 179 (1): 71-76.
44. The proteins content, of high quality, is about 18% of the blood (see: Ockerman H. W., C. L. Hansen (2000), ibid., pp. 325-353).

peptides that offer other health benefits such as analgesic and antinociception activities, have also been isolated from blood proteins.[45]

Iron is a mineral that is necessary for producing red blood cells and for redox processes.[46] Iron deficiency is the most common nutritional deficiency in the world since it affects about 20% of the world's population, with children and women at greater risk.[47] The primary cause of iron deficiency, apart from iron loss caused by blood loss through parasite infestation (for example, hookworm), is the inability to satisfy iron requirements with dietary iron[48] intake. In the poorer regions of the world (especially Asia and Africa) iron is provided mainly by plant-based diets that usually contain non-heme iron, a form of iron that is poorly absorbed.[49] Lack of iron in diets can cause many problems, may lead to unusual tiredness, shortness of breath, a decrease in physical performance,[50] and learning problems in children and adults, and may increase probability of getting an infection. In adults, low iron levels impair the ability to do physical work.[51] Iron deficiency, on the other hand, can proceed to iron-deficiency anemia and other adverse conditions, including at-risk pregnancy outcomes and, possibly, impaired intellectual development in infancy.[52]

Blood is a good source of bioavailable iron. As is well known, cooking decreases the bioavailability of iron and its absorption is improved when delivered in a meat-based diet: during digestion of meat, ferric iron can be converted into the bioavailable ferrous form.

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45. See: Teschemacher H. (2003), "Opioid Receptor Ligands Derived from Food Proteins", *Current Pharmaceutical Design*, 9 (16): 1331-1344; Gomes I., C. S. Dale, K. Casten, M. A. Geigner, F. C. Gozzo, E. S. Ferro, A. S. Heimann, L. A. Devi (2010), "Hemoglobin-derived peptides as novel type of bioactive signalling molecules", *AAPS Journal*, 12 (4): 658-669; Coderre T. J., I. Van Empela (1994), "The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests", *Pain*, 59 (3): 345–352; Aslaniants Zh. K., G. R. Melik-Eganov, A. V. Evstratov, M. P. Ivanov, S. G. Batrakov, N. V. Korobov, V. V. Iasnetsov (1991), "The effect of blood serum proteins from the seal on the analgetic action of narcotic analgesics", *Bulleten Eksperimentalnoi Biologii i Meditsiny* (Moskva), 112 (11): 503-5.

46. Lieu P. T., M. Heiskala, P. A. Peterson, Y. Yang (2001), "The roles of iron in health and disease", *Molecular Aspects of Medicine*, 22 (1-2): 1-87.

47. Assessing the iron status of populations. Report of a Joint World Health Organization, Centers for Disease Control and Prevention, Technical Consultation on the Assessment of Iron Status at the Population Level, 6-8 April 2004, Geneva, Switzerland; Martínez-Navarrete N., M. M. Camacho, J Martínez-Lahuerta, J. Martínez-Monzó, P. Fito (2002), "Iron deficiency and iron fortified foods – a review", *Food Research International*, 35 (2-3): 225–231.

48. Hoppe M., L. Hulthén, L. Hallberg (2008), "The importance of bioavailability of dietary iron in relation to the expected effect from iron fortification", *European Journal of Clinical Nutrition*, 62 (6): 761-769.

49. Chiplonkar S. A., K. V. Tarwadi, R. B. Kavedia, S. S. Mengale, K. M. Paknikar, V. V. Agte (1999), "Fortification of vegetarian diets for increasing bioavailable iron density using green leafy vegetables", *Food Research International*, 32 (3): 169-174; Yi-Chia Huang (2000), "Nutrient intakes and iron status of vegetarians", *Nutrition*, 16 (2): 147-148.

50. Gardner G. W., V. R. Edgerton, B. Senewiratne, R. J. Barnard, Y. Ohira (1977), "Physical work capacity and metabolic stress in subjects with iron deficiency anemia", *The American Journal of Clinical Nutrition*, 30 (6): 910–917.

51. Haas J. D., T. Brownlie (2001), "Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship", *Journal of Nutrition*, 131 (2S-2): 676S-690S.

52. Baynes R. (1990), "Iron Deficiency", *Annual Review of Nutrition*, 10 (1): 133-148.

Several strategies are commonly used to combat iron deficiency, including supplementation with capsules and tablets and fortification of processed foods.[53] However, in developing countries, food-based strategies remain the most sustainable approach for addressing iron and other micronutrient deficiencies.[54]

Fortifying staple foods with heme-iron, which is better absorbed than non-heme iron[55] because its absorption is essentially unaffected by other dietary factors, has therefore been suggested as a measure to overcome the problem of iron deficiency.

As bovine blood has the largest amount of heme iron than any animal source and is readily available, it has been suggested for the fortification of staple foods. Hertrampf and others[56] field tested an extruded rice flour, fortified with a bovine hemoglobin concentrate (Fe:14 mg/100 g of powder). Their results demonstrate that the consumption of a hemoglobin-fortified cereal is effective in markedly reducing the incidence of iron deficiency in breast-fed infants. Walter and others,[57] investigated the effect of bovine-hemoglobin-fortified cookies on the iron status of school children in a nationwide school lunch program in Chile. According to Haschke and others[58] feeding infants iron-fortified (12-15 mg/l) formulas is an effective and convenient means to protect infants from iron deficiency. Quintero-Gutierrez and others[59] evaluated the bioavailability of heme iron added to biscuit filling. It comprised two stages: first, the development of the heme iron enriched biscuit filling, second, the evaluation of the bioavailability of the mineral in fattening piglets. The heme iron was prepared

53. Suparat Reungmaneepaitoon, Chomdao Sikkhamondhol, Chansuda Jariyavattanavijit, Chowladda Teangpook (2008), "Development of instant noodles from high-iron rice and iron-fortified rice flour", *Songklanakarin Journal of Science and Technology*, 30 (6): 713-721.

54. FAO and ILSI, Preventing micronutrient malnutrition a guide to food-based approaches - Why policy makers should give priority to food-based strategies, Washington, DC 1997.

55. The chemical state of iron are ferrous ($^{+}Fe_2$) that is more easily absorbed than ferric ($^{+}Fe_3$) and this change is largely dependent on the presence of luminal gastric acid and ceruloplasmin within the intestinal mucosal cell. Heme and non-heme are two forms of dietary iron. The difference between heme and non-heme iron is that the first is derived from haemoglobin while non-heme irons do not come from an animal source. Since the iron in animal-based foods is about 40% heme iron and 60% non-heme iron, animal-based foods are good sources of absorbable iron (See: Hallberg L., L. Hulthén (2000), "Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron", *The American Journal of Clinical Nutrition*, 71 (5): 1147-60.

56. Hertrampf E., M. Olivares, F Pizarro, T. Walter, M. Cayazzo, G. Heresi, S. Llaguno, P. Chadud, A. Stekel (1990), "Haemoglobin fortified cereal: a source of available iron in breast fed infants", *European Journal of Clinical Nutrition*, 44 (11): 793-798; Kikafunda J. K., P. Sserumaga (2005), "Production and use of a shelf-stable bovine blood powder for food fortication as a food-based strategy to combat iron deficiency anaemia in Sub-Saharan Africa", *African Journal of Food Agriculture Nutrition and Development*, 5 (1): 1-18.

57. Walter T., E. Hertrampf, F. Pizarro, M. Olivares, S. Llaguno, A. Letelier, V. Vega, A. Stekel (1993), "Effect of Bovine-Hemoglobin Fortified Cookies on Iron Status of Schoolchildren - a Nationwide Program in Chile", *The American Journal of Clinical Nutrition*, 57 (2): 190-194.

58. Haschke F., H. Vanura, C. Male, G. Owen, B. Pietschnig, E. Schuster, E. Krobath, C. Huemer (1993), "Iron nutrition and growth of breast- and formula-fed infants during the first 9 months of life", *Journal of Pediatric Gastroenterology and Nutrition*, 16 (2): 151-6.

59. Quintero-Gutiérrez A. G., G. González-Rosendo, J. Sánchez-Muñoz, J. Polo-Pozo, J. J. Rodríguez-Jerez (2008), "Bioavailability of heme iron in biscuit filling using piglets as an animal model for humans", *International Journal of Biological Science*,. 4 (1): 58-62.

by isolating hemoglobin from porcine blood followed by enzyme hydrolysis and subsequent separation of globin from the heme group using ultrafiltration. They showed that an acceptable product with high heme iron content can be formulated and suitable for use as biscuit filling. The heme iron supplement produced weight increase and a lessened mortality in fattening pigs. A similar study was conducted by González-Rosendo and others.[60] They used a heme-iron concentrate product derived from swine hemoglobin to enrich the chocolate-flavored filling of biscuits and the bioavailability of this source of heme-iron was assessed in adolescent girls. The study showed that the iron contained in the heme-iron concentrate was well absorbed and tolerated by the adolescents included in the study. Similarly Olivares and others support the study[61]. During their research school-age children in Chile received 30 g of wheat-flour biscuits daily through a "National School Lunch Program". To improve iron nutrition, the biscuits were fortified with a 6% bovine hemoglobin concentrate. The high-iron bioavailability, the good organoleptic characteristics and the biological effect on iron nutritive made the product an appealing alternative to combat iron deficiency.

However, some[62] believe that it is impractical to use hemoglobin as an iron supplement because it is low in iron. Vaghefi and others[63] assert at the end of their study that heme iron absorption depends not only on its solubility but also relies mainly on the balance between the strength of heme-peptides and the polymerization rate of heme. Others[64] state that heme-iron is poorly soluble at low gastric pH, and its absorption would be less than iron absorption from muscle.[65] Hallberg and others[66] observe that since Ca inhibits the absorption of heme- and non-heme-Fe to the same extent, their results strongly suggest that Ca interferes with the transport of Fe through the mucosal cell and, at a late stage, is common for heme- and non-heme-Fe transport. They conclude that observations of Ca strong

60. González-Rosendo G., J. Polo, J. J. Rodríguez-Jerez, R. Puga-Díaz, E. G. Reyes-Navarrete, A. G. Quintero-Gutiérrez (2010), "Bioavailability of a heme-iron concentrate product added to chocolate biscuit filling in adolescent girls living in a rural area of Mexico", *Journal of Food Science*, 75 (3): 73H-78H.
61. Olivares M., E. Hertrampf, F. Pizzarro, T. Walter, M. Cayazzo, S. Llaguno, P. Chadud, N. Cartagena, V. Vega, M. Amar, A. Stekel (1990), "Hemoglobin-fortified biscuits: bioavailability and its effect on iron nutriture in school children", *Archivos latinoamericanos de nutrición*, 40 (2): 209-220.
62. For review see: West A. R., P. S. Oates (2008), "Mechanisms of heme iron absorption: Current questions and controversies", *World Journal of Gastroenterology*, 14 (26): 4101-4110.
63. Vaghefi N., F. Nedjaoum, D. Guillochon, F. Bureau, P. Arhan, D. Bouglé (2002), "Influence of the extent of hemoglobin hydrolysis on the digestive absorption of heme iron. An in vitro study", *Journal of Agricultural and Food Chemistry*, 50 (17): 4969-4973.
64. Mackenzie B., M. D. Garrick (2005), "Iron Imports. II. Iron uptake at the apical membrane in the intestine", *American Journal of Physiology*, 289 (6): G981-G986; Hallberg L. (1981), "Bioavailability of Dietary Iron in Man", *Annual Review of Nutrition*, vol. 1: 123-147; Sarker S. A., L. Davidsson, H. Mahmud, T. Walczyk, R. F. Hurrell, N. Gyr, G. J. Fuchs (2004), "Helicobacter pylori infection, iron absorption, and gastric acid secretion in Bangladeshi children", *The American Journal of Clinical Nutrition*, 80 (1): 149–153.
65. Monsen E. R. (1988), "Iron nutrition and absorption: dietary factors which impact iron bioavailability", *Journal of the American Dietetic Association*, 88 (7): 786-90; Pallares I., M. S. Campos, I. López-Aliaga, M. Barrionuevo, A. E. Gómez-Ayala, M. J. Alférez, S. Hartiti, F. Lisbona (1996), "Supplementation of a cereal-milk formula with haem iron palliates the adverse effects of iron deficiency on calcium and magnesium metabolism in rats", *Annals of Nutrition and Metabolism*, 40 (2): 81-90.
66. Hallberg L., L. Rossander-Hulthèn, M. Brune, A. Gleerup (1993), "Inhibition of haem-iron absorption in man by calcium", *British Journal of Nutrition*, 69 (2): 533-540.

interference of the absorption of both heme- and non-heme-Fe have important nutritional implications.

Also the antioxidant activity of blood protein peptides has been studied and demonstrated. Xu and others[67] investigated the antioxidant activity of porcine plasma hydrolysate obtained by pepsin and papain digestion in a peroxidation system of aqueous linoleic acid. Both the pepsin and papain digested hydrolysates exhibited significant activities against linoleic acid oxidation and good DPPH free radical scavenging ability. Blood has the potential to serve as a useful source of valuable peptides with antihypertensive, antioxidant, and antibacterial properties. Several bioactive peptides that offer other health benefits such as analgesic and antinociception (reduction in pain sensitivity) activities have also been isolated from blood proteins. Gomes and others[68] provide more detailed information on some of these peptides that have been isolated from blood. They review the status of the hemoglobin (Hb) peptide field and highlight recent reports on the identification of a molecular target for a novel set of Hb peptides, hemopressins, and the implication of these peptides to normal cell function and disease. The potential therapeutic applications for these Hb-derived hemopressin peptides are also discussed.

Binders or meat glues

Traditionally, binders have been used in meat products to counter the textural and sensorial changes brought about by processing. Binders have a macromolecular structure that has the capacity to form matrices that retain aroma and nutrients and also entrap large amounts of water.[69] Devadason and others[70] point out that binders or glues are used to bind water and fat to stabilize meat emulsions in ground meat products. A particular kind of glue is represented by a powder substance (meat glue) made from an animal blood-clotting agent (a transglutaminase enzyme) that connects small pieces of meat. Jacquay et al.[71] have investigated the modifications, as desirability and strength of bind, of meatloaf caused by different hamburger binders: powdered skim milk, egg binding and powdered soy milk.

67. Xu X., R. Cao, L. He, N. Yang (2009), "Antioxidant activity of hydrolysates derived from porcine plasma", *Journal of the Science of Food and Agriculture*, 89 (11): 1897–1903; Álvarez C., M. Rendueles, M. Díaz (2012), "Production of Porcine Hemoglobin Peptides at Moderate Temperature and Medium Pressure under a Nitrogen Stream. Functional and Antioxidant Properties", *Journal of Agricultural and Food Chemistry*, 60 (22): 5636-5643.

68. Gomes I., C. S. Dale, K. Casten, M. A. Geigner, F. C. Gozzo, E. S. Ferro, A. S. Heimann, L. A. Devi (2010), ibid. *AAPS Journal*, 12 (4): 658-669.

69. Chen M. J., C. W. Lin (2002), "Factors affecting the water-holding capacity of fibrinogen/plasma protein gels optimized by response surface methodology", *Journal of Food Science*, 67 (7): 2579-2582.

70. Devadason I. P., A. S. R. Anjaneyulu, Y. Babji (2010), "Effect of different binders on the physicochemical, textural, histological, and sensory qualities of retort pouched buffalo meat nuggets", *Journal of Food Science*, 75 (1): S31-S35. They studied the functional properties of 4 binders, namely corn starch, wheat semolina, wheat flour, and tapioca starches, in order to assess their ability to improve the quality of buffalo meat nuggets processed in retort pouches. Incorporation of corn starch in buffalo meat nuggets produced more stable emulsion than other binders used. More recently, have investigated the modifications, as desirability and strenght of bind, of meatloaf caused by different hamburger binders: powdered skim milk, egg binding and powdered soy milk.

71. Jacquay A., C. Beaver, S. Fisher (2011), "Alternate hamburger binding methods and their effects on flavour", *Food Chemistry* FN 453, Research Project-Fall (www.cfs.purdue.edu/fn/fn453/).

Transglutaminases are a family of proteins that are widely distributed in all living organisms. They are enzymes needed in living animal organisms to repair lesions of body tissues and create stable structures by extensively cross-linking protein molecules.[72] There has been a recent surge in findings supporting that dysregulated expression and function of transglutaminases contribute to pathological conditions, such as neurodegenerative disorders, tissue fibrosis, cancer metastasis, celiac disease, and diseases related to defective assembly of the stratum corneum of the skin.

The TGM1 gene[73] ("transglutaminase-1"), that provides instructions for making an enzyme called transglutaminase-1, is found in cells that make up the outermost layer of the skin (the epidermis). The transglutaminase-1 enzyme is involved in the formation of the cornified cell envelope, which is a structure that surrounds skin cells and protects against water loss and infection. The cornified cell envelope is made up of multiple proteins that are linked to one another (crosslinked). The crosslinking of these proteins is facilitated by the transglutaminase-1 enzyme that is essential for the assembly of the cell envelope barrier in stratified squamous epithelia. It is usually bound to membranes, but to date most studies with it have involved solution assays.[74]

It was also found that the "Transglutaminase-2" (TG2), defined as an enigmatic enzyme with diverse functions[75] and as a molecular Swiss army knife,[76] is an extremely versatile protein exhibiting transaminating, protein disulphide isomerase and guanine and adenine nucleotide binding and hydrolyzing activities. TG2 can also act as a protein scaffold or linker. This unique protein also undergoes extreme conformational changes, exhibits localization diversity and its multiple biochemical activities account for its involvement in a wide variety of cellular processes encompassing differentiation, inflammation, cell death, cell migration, and wound healing.

Transglutaminase-3 (TG3), also known as epidermal Transglutaminase (Tgase E), belongs to the family of Transglutaminase enzymes that catalyze the posttranslational modification of proteins via calcium dependent cross-linking reactions. TG3 is involved in the formation of the cornified envelope in skin keratinocytes.[77]

72. Greenberg C. S., P. J. Birckbichler, R. H. Rice (1991), "Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues", *The FASEB Journal*, 5 (15): 3071-7.

73. K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase. See: Kim I. G., O. W. McBride, M. Wang, S. Y. Kim, W. W. Idler, P. M. Steinert (1992), "Structure and organization of the human transglutaminase 1 gene", *The Journal of Biological Chemistry*, 267 (11): 7710–7; Grenard P., M. K. Bates, D. Aeschlimann (2001), "Evolution of transglutaminase genes: identification of a transglutaminase gene cluster on human chromosome 15q15. Structure of the gene encoding transglutaminase X and a novel gene family member, transglutaminase Z", *The Journal of Biological Chemistry,* 276 (35): 33066–78.

74. Nemes Z., L. N. Marekov, P. M. Steinert (1999), "Involucrin Cross-linking by Transglutaminase 1. Binding to membranes directs residue specificity", *The Journal of Biological Chemistry*, 274 (16): 11013-11021.

75. Fesus L., M. Piacentini (2002), "Transglutaminase 2: an enigmatic enzyme with diverse functions", *Trends in Biochemical Sciences*, 27 (10): 534-9.

76. Gundemir S., G. Colak, J. Tucholski, G. V. Johnson (2012), "Transglutaminase 2: a molecular Swiss army knife", *Trends in Biochemical Sciences*, 1823 (2): 406-19.

77. Zhang J., H. Y. Zhi, F. Ding, A. P. Luo, Z. H. Liu (2005), "Transglutaminase 3 expression in C57BL/6J mouse embryo epidermis and the correlation with its differentiation", *Cell Research*, 15 (2): 105-10.

The recently introduced synthetic form of these substances develop similar effects in meat as they have the capacity to form bonds between superficial protein structures of individual muscle meat pieces. Transglutaminase enzymes act only on proteins by catalyzing reactions in the formation of covalent bonds between a carboxylamide group of the lateral chain on a glutamine residue and an amino group of the lateral chain of a Lysine. These bonds may be formed between proteins of distinct types and origin, as myosins, caseins, soya globulins, glutens, actins, etc.

As a consequence transglutaminases may be used[78] in various meat processing phases for bonding a diverse range of food materials such as meat, poultry, dairy, and seafood; from tumbling and reconstituting cooked hams; to creating protein network structures. Transglutaminase enzymes have an impact on specific meat processing technologies: tumbling procedures could be shortened, the utilization of phosphates and other binding substances reduced or completely substituted, and the confection of sausages without membranes, etc. Even the presence of transglutaminases in raw-fermented sausages, formed by a mix of coarsely chopped meat and fat particles, can be strengthened by the cohesion of such particles during ripening. By binding thin cuts of meat together, one can obtain bigger pieces. Irregular cuts can be "glued" together into a more regular shape and permit even cooking. In this way it can be useful to glue chicken or bacon or fish skin onto other types of meat and fish. The enzyme enables food processors to adhere various types of meat together, for example, as in imitation crabmeat.

British chef Blumenthal[79] is credited with the introduction of transglutaminases[80] into modern cooking. He uses a white powder made by "taking the clotting agents out of pigs" and cows "blood" in order to clot together chunks of meat.[81]

Parés et al[82] have studied the properties of gels obtained from porcine blood plasma under different pH conditions. Gels from liquid and spray-dried plasma were prepared and analyzed for water holding capacity (WHC), texture, and microstructure at pH 7.4, 6, 5.5 and 4.5. At low acid pH, such as that of fermented products, plasma is not effective as a gelling agent producing soft and exuding gels. The penetration force of the gel from dehydrated plasma was always lower than that prepared from liquid plasma where the pH was the same, but neither the WHC nor the microstructure of gels were affected.

78. Motokia M., K. Segurob (1998), "Transglutaminase and its use for food processing", *Trends in Food Science & Technology*, 9 (5): 204–210.

79. Heston Blumenthal owns "The Fat Duck Restaurant", awarded three Michelin stars, restaurant of the year, and considered best restaurant in the world and best restaurant in the UK.

80. Yokoyama K., N. Nio, Y. Kikuchi (2004), "Properties and applications of microbial transglutaminase", *Applied Microbiology and Biotechnology*, 64 (4): 447–454.

81. The process begins with sprinkling a teaspoon of powdered transglutaminase on various meat scraps (sometimes lesser-quality meat) and binding them together with the hands. Roll them up in plastic wrap. Put in the fridge and 6 hours later, you have an easily-sliced piece of meat that looks like filet mignon. The meat glue, that has been used in American restaurants for decades, is USDA-approved and considered safe for the diner. The problem lies with the increased potential for bacteria in meat that has been created by multiple pieces. The meat glue additive was approved formally in Europe in 2010, but one year later the arguments against its use are growing.

82. Parés D., E. Saguer, J. Saurina, J. J. Suño, C. Carretero (1998), "Functional Properties of Heat Induced Gels from Liquid and Spray-Dried Porcine Blood Plasma as Influenced by pH", *Journal of Food Science*, 63 (6): 958-961.

Every country in Europe, in recent years, first approved, then banned, and then permitted the use of meat glue or transolutaminases, called also thrombian. In May 19, 2010, the European Parliament voted to ban the use of a kind of glue[83] as it believes that there is "a clear risk that meat containing thrombin would find its way into meat products served in restaurants and other public establishments serving food, given the higher prices that can be obtained for pieces of meat served as a single meat product". Legislators considered that consumers should be able to trust that the meat they are buying is a real steak and not simply pieces of meat glued together. The ban, however, never took effect, as in accordance with Commission directive 2010/67/EU,[84] and meat glue is permitted for use as a food additive for reconstituting food.

In the USA, this binder is permitted in products such as blood sausage, blood pudding, blood soup, and in beef patties.[85] "Beef fibrin" defined as "a component mixture of beef fibrinogen and beef thrombin plasma protein used to bind pieces of meat or poultry together" is permitted up to 10%, provided it is labeled as required.[86]

In addition, in the USA, a coating of beef blood is permitted on cured products (e. g. ham, hamette, etc.), and doubts about its usage, such as those expressed in the EU, have not been an issue.

Blood flours

Blood flour is dried animal blood, typically cow blood, but it can also be the blood of any animal that goes through a meat packing plant. Blood is often collected in conjunction with the slaughtering of animals and subsequently used as a supplemental protein source for livestock. Normally it is dried and made into blood meal so that it can be handled and incorporated into feed more easily. The blood is collected after the animals are killed and then dried to make a powder.

In practice, the withdrawal of blood necessitates a specially designed hollow knife, sterilized between each sticking operation, to which a hose is attached. The hose allows the blood to flow into a container. After bleeding, blood clots in 3-10 minutes. This clotting is caused by thrombin, which converts soluble fibrinogen in the blood into insoluble fibrin. Anticoagulants are usually added in the hollow knife. The anticoagulants normally used are either trisodium citrate (sodium citrate), or citric acid, or a mixture of phosphates, or sodium hexameta phosphate, or sodium acid pyrophosphate, or heparin[87] or oxalates.[88]

83. A Nebraska (USA) based company produces a kind of binder that consists of two components: *Fibrinogen* i.e. fibrinogen enriched plasma, and *Thrombin*, an enzyme which initiates the gelling and the subsequent binding process of meat or fish pieces.

84. Commission Directive 2010/67/EU of 20 October 2010, amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners (Official Journal of the European Union, L. 277/17, 21.10.2010).

85. See: Tartè R. (2009) (Edtr.), Ingredient in Meat Products: Properties, Functionality and Applications, Springer, New York, pp. 163-4.

86. USDA/FSIS, (2005), *Food Standards and Labeling Policy Book*. Available: http://www.fsis.usda.gov/OPPDE/larc/ Policies/Labeling_ Policy_Book_082005.pdf

87. Heparin is a natural blood component that helps prevent coagulation in the live animal during blood circulation. Commercially, it is available in the sodium, lithium, or calcium salt, and it inhibits the formation of thrombin from prothrombin.

88. Oxalate is poisonous and can not be used for blood which used for food or pharmaceutical uses.

Normal bleeding time used in industry is 6' for cattle, 4-5' for sheep, 3-4' for calves, and 6' for pigs. The quantity of blood collected during bleeding in the slaughter operation is approximately 50% of the total present in the body since the remaining blood (50%) is retained in the body's capillary system.

Animal blood is either spray dried as regular whole blood or, after separation, into plasma and red albumin. Normally blood is dried using one of the following methods: drum, ring, flash or spray dried.[89] The drying method used has an effect on the digestibility of the crude protein (CP) of the blood meal, because there is a direct relationship between the amount of heat applied and the digestibility of the CP.[90] As the amount of heat applied increases the digestibility of the CP decreases.[91]

Blood proteins

Protein malnutrition is a great problem in poor and in developing countries, especially during the transitional phase of weaning in infants, as it retards their physical and mental development. Kwashiorkor,[92] more common in very poor countries of some parts of the world, has been linked to the quality and quantity of protein in maize when it is the sole source of protein for infants[93].

Blood protein may be used as a potential source of large quantities of dietary protein, because it is well balanced in amino acid composition. Since the blood protein is abundant,[94]

89. Cook E. M., H. D. DuMont (1971), *Process Drying Practice*, McGraw-Hill Publishing Co., New York; Williams-Gardner A. (1991), *Industrial Drying*, Leonard Hill Books, London.

90. Waibel P. E., M. Cuperlovic, R. F. Hurrell, K. J. Carpenter (1977), "Processing damage to lysine and other amino acids in the manufacture of blood meal", *Journal of Agricultural and Food Chemistry*, 25 (1): 171-175.

91. Kats L. J., J. L. Nelssen, M. D. Tokach, R. D. Goodband, T. L. Weeden, S. S. Dritz, J. A. Hansen (1994), "The effects of spray-dried blood meal on growth performance of the early-weaned pig", *Journal of Animal Science*, 72 (8): 2075-2081.

92. "Kwashiorkor" is a form of malnutrition that occurs when there is not enough protein in the diet. It often occurs during political unrest, a drought or other natural disaster. These conditions are responsible for a lack of food, which leads to malnutrition. It is very rare in children in the UE, United States and in other countries as Japan, South Korea, Australia , etc. where the diet is rich in protein.

93. Of this opinion: Cantor S. M., H. J. Roberts (1967), "Improvement in protein quality in cornbased foods", *Cereal Science Today*, 12: 443-445, 460-462; Banigo E. O. I., A. I. Adeyemi (1975), *A comparative study of the commercial practice of traditional ogi manufacture using high-lysine (opaque-2) corn and normal corn*, Proceedings of the 10th International Congress of Nutrition, Kyoto, Japan, p. 402; Akinrele I. A., C. C. A. Edward (1971), "An assessment of the nutritive value of maize soya mixture. «Soya-ogi» as a weaning food in Nigeria", *British Journal of Nutrition*, 26 (2): 177-185; Makinde M. A., P. A. Lachange (1989), "Optimization of protein nutritive value of ogi", *Nigerian Journal of Nutrition Science*, 10: 85-93; Mottern H. H., T. S. De Buckle, C. Pardo (1970), "Protein enrichment of Colombian corn cakes", *Cereal Science Today*, 15: 108-112; FAO, *Maize in Human Nutrition, Food and Agriculture Organization of the United Nations*, FAO Food and Nutrition Series, n. 25,1992, pp. 81-131.

94. It has been estimated that only in China about 1,5 millions of tons of porcine blood, produced annually until 2006, has a proteine content equivalent to that 2 millions of tons of meat (See: Wang J. Z., M. Zhang, F. Z. Ren, B. Z. Han, L. Wang, S. W. Chen, A. Humera (2007), "Changes of chemical and nutrient composition of porcine blood during fermentation by Aspergillus oryzae", *World Journal of Microbiology and Biotechnology*, 23: 1393–1399). In Italy we have estimated that the annual production of bovine blood is about 40,000 t and of porcine blood is roughly 150,000 t.

cheap, readily available, and has a proven track record in animal nutrition, it has been suggested for use as protein supplements in infant formula to tackle protein malnutrition.

Five experiments were conducted by Pierce and others,[95] to evaluate the effects of dietary spray-dried porcine plasma (SDPP) and spray-dried bovine plasma (SDBP) and their various molecular weight fractions on performance of pigs weaned at approximately 14-21 days of age. These studies verified that the immunoglobulins, primarily immunoglobulin G, are the major components in plasma that stimulates growth in early-weaned pigs. Similar results have been observed in newly weaned mice,[96] indicating that the benefits of including blood proteins in the diet are not species-specific. Thomson and others conclude that mice respond to dietary inclusion of spray-dried porcine plasma protein (SDPP) with increases in average daily feed intake (ADFI), Average daily gain (ADG), and gain-to-feed ratio (G/F) during the period immediately after weaning may, therefore, serve as appropriate models for pig responses to SDPP.

The adequacy of blood proteins as protein supplements has also been demonstrated by Oshodi and others,[97] who investigated in vitro multienzyme protein digestibility of an infant-weaning food produced from maize flour and bovine blood proteins using the pH-stat and pH-drop procedures. Their results indicated an improved digestibility when blood protein concentrate was added to the maize. After blending with the blood protein concentrate, the level of available iron was double that of maize flour alone. The bovine blood powder concentrate used was so bland in taste that its addition to the maize flour did not alter the overall flavor.

Recent physiological and biochemical research has shown that the protein in food not only furnishes amino acids but also provides bioactive peptides after digestion or food processing. Clare and Swaisgood[98] have reviewed the scientific literature and attempted to stimulate consideration of the continued use of bioactive peptides and their expanded development as a commercial product. Several applications have already evolved. Potentially, the addition of bioactive peptides to food products could improve consumer safety as a result of their antimicrobial properties. Bioactive peptides may function as health care products, providing therapeutic value for either treatment of infection or prevention of disease. As a consequence, bioactive peptides produced from both plant and animal sources are being investigated and have been reported to have antinematodal,[99] antiviral and antibacterial,[100]

95. Pierce J. L., G. L. Cromwell, M. D. Lindemann, L. E. Russell, E. M. Weaver (2005), "Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs", *Journal of Animal Science*, 83 (12): 2876-2885.

96. Thomson J. E., E. E. Jones, E. J. Eisen (1994), "Effect of spray-dried porcine plasma protein on feed intake, growth rate, and efficiency of gain in mice", *Journal of Animal Science*, 72 (10): 2690-2695.

97. Oshodi A. A., R. M. Beames, S. Nakai (1997), "In vitro protein digestibility, amino acid profile and available iron of infant weaning food prepared from maize flour and bovine blood", *Food Research International*, 30 (3/4): 193-197.

98. Clare D. A., H. E. Swaisgood (2000), "Bioactive milk peptides: a prospectus", *Journal of Dairy Science*, 83 (6): 1187-1195.

99. The antinematodal activity and mechanism of a 23-mer antimicrobial peptide, PMAP-23, derived from pig myeloid was investigated by Yoonkyung Park, Seung-Hwan Jang, Dong Gun Lee, Kyung-Soo Hahm (2004), (in "Antinematodal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against Caenorhabditis elegans", *Journal of Peptide Science*, 10 (5): 304–311).

100. Jenssen H., P. Hamill, R. E. W. Hancock (2006), "Peptide Antimicrobial Agents", *Clinical Micro-*

opioid,[101] antioxidant,[102] antitumor,[103] and angiotensin I-converting enzyme (ACE) inhibitory (anti-hypertensive)[104] activities.

The studies previously listed support the use of bovine blood proteins as efficient protein supplements in cereal based weaning and infant diets as a measure to tackle protein malnutrition problems.

The suspicion that blood provides a haven for pathogens and toxic metabolites is a strong reason why some people avoid consuming blood. It is however necessary to remember, that in general, foods of animal origin are easily contaminated with microorganisms, often pathogens, due to poor handling and processing.[105]

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biology Review, 19 (3): 491-511; Lee D. G., D.-H. Kim, Y. Park, H. K. Kim, H. N. Kim, Y. K. Shin, C. H. Choi, K. –S. Hahm (2001), "Fungicidal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against Candida albicans", *Biochemical and Biophysical Research Communications*, 282: 570-574; Yu P. L., S. D. Choudhury,, K. Ahren (2001), "Purification and characterization of the antimicrobial peptide, ostricacin", *Biotechnology Letters*, 23 (3): 207–210.

101. Perpetuo E. A., L. Juliano, I. Lebrun (2003), "Biochemical and pharmacological aspects of two bradykinin-potentiating peptides obtained from tryptic hydrolysis of casein", *Journal of Protein Chemistry*, 22 (7/8): 601-606.

102. Tri Agus Siswoyo, Eka Mardiana, Kyun Oh Lee, Keizo Hoshokawa (2011), "Isolation and Characterization of Antioxidant Protein Fractions from Melinjo (Gnetum gnemon) Seeds", *Journal of Agricultural and Food Chemistry*, 59 (10): 5648-5656; Se-Kwon Kim, Yong-Tae Kim, Hee-Guk Byun, Kyung-Soo Nam, Dong-Sik Joo, F. Shahidi (2001), "Isolation and characterization of antioxidative peptides from gelatine hydrolysate of Alaska pollack skin", *Journal of Agricultural and Food Chemistry*, 49 (4): 1984-199; Je-Ruei Liu, Ming-Ju Chen, Chin-Wen Lin (2005), "Antimutagenic and antioxidant properties of milk-kefir and soymilk-kefir", *Journal of Agricultural and Food Chemistry*, 53 (7): 2467-2474; Pratt D. E., C. Di Pietro, W. L. Porter, J. W. Giffee (1982), "Phenolic antioxidants of soy protein hydrolyzates", *Journal of Food Science*, 47 (1): 24-35; Esaki H., R. Watanabe, H. Onozaki, S. Kawakishi, T. Osawa (1999), "Formation mechanism for potent antioxidative o-dihydroxyisoflavones in soybeans fermented with Aspergillus saitoi", *Bioscience, Biotechnology, and Biochemistry*, 63 (5): 851-858; Chen H. M., K. Muramoto, F. Yamauchi, K. Nokihara (1996), "Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein", Journal of Agricultural and Food Chemistry, 44 (9): 2619-2623; Tong L. M., S. Sasaki, Julian D. McClements, E. A. Decker (2000), "Mechanisms of the antioxidant activity of a high molecular weight fraction of whey", *Journal of Agricultural and Food Chemistry*, 48 (5): 1473-1478.

103. Zeiger E. (1987), "Carcinogenicity of mutagens: Predictive capability of the Salmonella mutagenesis assay for rodent carcinogencity", *Cancer Research*, 47 (5): 1287-1296; Qureshi A., P. L. Colinb D. J. Faulkner (2000), "Microsclerodermins F–I, antitumor and antifungal cyclic peptides from the lithistid sponge Microscleroderma sp.", *Tetrahedron*, 56: 3679–3685; Shiomi M., K. Sakaki, M. Murofushi, K. Aibara (1982), "Antitumor activity in mice of orally administered polysaccharide from kefir grain", *Japanese Journal of Medical Science & Biology*, 35: 75-80.

104. Matsui T., Akiko Yukiyoshia, Shima Doib, Hiroyuki Sugimotob, Hideo Yamadab, Kiyoshi Matsumotoa (2002), "Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR", *The Journal of Nutritional Biochemistry*, 13 (2): 80-86; Arihara K., Y. Nakashima, T. Mukai, S. Ishikawa, M. Itoh (2001), "Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins", *Meat Science*, 57 (3): 319–324; Kapela R., A. Chabeaua, J. Lesagec, G. Rivierec, R. Ravallec-Plea, D. Lecouturiera, M. Wartelled, D. Guillochona, P. Dhulster (2006), "Production, in continuous enzymatic membrane reactor, of an anti-hypertensive hydrolysate from an industrial alfalfa white protein concentrate exhibiting ACE inhibitory and opioid activities", *Food Chemistry*, 98 (1): 120–126.

105. Al-Bachir M., A. Mehio (2001), "Irradiated luncheon meat: microbiological, chemical and sen-

Manufacturers and processors have instituted measures to guarantee the safety of these blood proteins. In the UE, article 10 of Regulation (EC) No 1069/2009 of 21 October 2009 and in the US, Federal Regulation 9 CFR 310.20 monitors blood that enters the food chain, ensuring that it originates from official establishments whose livestock and carcasses have passed inspection.

Blood sausages

The traditional use of blood, however, is generally restricted to such products as blood sausages where the black color is both expected and acceptable. When plasma is used in meat sausages, because of its gelling properties, it will decrease shrinkage and increase yield, and the texture of the finished product becomes more rigid. Meat sausage, produced with emulsification of fat by plasma, is able to retain the fat during its heating.

Clarifiers

A clarifying or fining agent makes wine clear by removing proteins from the wine. The agents eventually settle out of the wine. Different proteins serve as clarifying agents depending upon both the type of wine and the desired flavor. Some clarifiers are animal-based products, while others are earth-based. Wine may be refined either with eggs, milk, or dried blood powder. Although blood of large mammals may serve as a clarifier in some old Mediterranean countries, its use is forbidden in wine from either the United States or Europe.

Curing agents

Curing is the treatment of muscle meat with common salt or sodium chloride (NaCl) and sodium nitrite ($NaNO_2$). In the past, curing was mainly applied to extend the storage life of entire pieces of muscle meat by using the preserving effects of common salt and to a lesser extent sodium nitrite. In modern meat processing, the storage life is less important since more efficient meat preservation methods, in particular cooling and freezing, are available, and curing is now applied to achieve a pink-red color as well as a typical flavor and taste in processed meat products.

Although sodium nitrite continues to be used as a common food additive, its use has come under criticism in recent years because it has a tendency to react with amines, amides, amino acids and related compounds present in the meat to produce carcinogenic nitrosoamine compounds. As a consequence, efforts are being made to reduce the amount of sodium nitrite used in the curing system and to develop alternative methods of curing that avoid the use of sodium nitrite altogether.[106]

Nowadays, no single capable agent has been identified to replace nitrite, as it is extremely difficult to find a single compound that can fully reproduce the multifunctional role of nitrite (as a colorant, antioxidant, and antimicrobial).[107] One of the colorants suggested for use

sory characteristics during storage", *Food Chemistry*, 75 (2): 169-175.

106. Pegg R. B., F. Shahidi, *Nitrite Curing of Meat: The N-Nitrosamine Problem and Nitrite Alternatives*, Food & Nutrition, Inc., Trumbull, Connecticut, USA, pp. 209-254.

107. Nollet L. M. L., F. Toldrá (Edts) (2006), *Advanced Technologies For Meat Processing*, CRC Press – Taylor & Francis Group, NW, pp. 309-328.

in a composite sodium nitrite-free cocktail for meat curing is Cooked Cured Meat Pigment (CCMP), prepared by reacting bovine or porcine red blood cells with a nitrosating agent.[108] According to Shahidi and Pegg,[109] the application of this CCMP in different formulations to comminuted and solid cuts of muscle foods has showed that color, oxidative stability and flavor of the treated samples were similar to those of their nitrite-cured counterparts. Shahidi et al.[110] have also investigated the effects of 5 and 10 kGy irradiation on the color and oxidative stability of meats treated with nitrite or a nitrite-free curing system.

Dyes and color enhancers

Color, as it is well known, is one of the main factors used by consumers when evaluating the quality and freshness of food products, especially meat products. The growing aversion to some artificial food dyes has resulted in the substitution of artificial colorants with natural ones. Stabilized hemoglobin (frozen or powder hemoglobin),[111] obtained from porcine or bovine blood, represents a good source of natural red colorant and is also an iron supplement for meat products in some countries. In order to stabilize hemoglobin or to prevent hemoglobin auto-oxidation during drying and subsequent storage, Salvador et al.[112] suggest the use of chelating agents, such as nicotinic acid (NA, 2% w/v) or nicotinamide (Nam, 2.5% w/v) along with glucose, as reducing agents (G, 10% w/v) which can be combined with fresh porcine hemoglobin in order to stabilize its red color during spray-drying and during powder storage at room temperature. These chelating agents have the ability to form complexes with the heme moiety. From the results, it can also be concluded that glucose is the main contributor to the color stabilization of the hemoglobin powder.

Mancini and Hunt[113] recently updated research regarding numerous ante-mortem and post-mortem factors that influence meat color. They noted that carbon monoxide saturation of hemoglobin resulted in conversion into the more stable carboxyhemoglobin and has been proposed as an alternative method to stabilize hemoglobin.

108. Shahidi F., R. B. Pegg (1990), "Colour characteristics of cooked cured-meat pigment and its application to meat", *Food Chemistry*, 38 (1): 61-68.
109. Shahidi F., R. B. Pegg (1992), "Nitrite-free meat curing systems: Update and review", *Food Chemistry*, 43 (3): 185–191.
110. Shahidi F., R. B. Pegg, K. Shamsuzzaman (1991), "Color and Oxidative Stability of Nitrite-Free Cured Meat after Gamma Irradiation", *Journal of Food Science*, 56 (5): 1450-1452.
111. Spray-drying haemoglobin is a good way to preserve the red blood cell fraction, better than freeze drying (See: Saguer E., S. Altarriba, C. Lorca, D. Parés, M. Toldra`, C. Carretero (2003), "Colour stabilization of spray-dried porcine red blood cells using nicotinic acid and nicotinamide", *Food Science and Technology International*, 9 (4): 301-307).
112. Salvador P., M. Toldrà, D. Parés, C. Carretero, E. Saguer (2009), "Color stabilization of porcine hemoglobin during spray-drying and powder storage by combining chelating and reducing agents", *Meat Science*, 83 (2): 328-333.
113. Mancini R. A., M. C. Hunt (2005), "Current research in meat color", *Meat Science*, 71 (1): 100–121. See also: Knock R. C., M. Seyfert, M. C. Hunt, M. E. Dikeman, R. A. Mancini, J. A. Unruh, J. J. Higgins, R. A. Monderen (2006), "Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks", *Meat Science*, 74 (2): 312–318.

Egg albumin substitute

Blood albumin is used as a substitute for egg albumin in food. Albumin is a type of globular protein that has a roughly spherical structure and is water-soluble. Many types can be found in the natural world, and two of the most familiar examples can be found in egg whites (known as ovalbumin) and in human blood. Albumin is vitally important to the health and well being of many organisms. It is an important component of life because it transports essential fatty acids from adipose tissue to muscle tissue. The protein also contributes to the regulation of osmosis, helping to transport hormones, drugs, and other substances through the blood.

When heated, albumin and other proteins tend to coagulate; they transform, recombining in a new configuration and turn white and opaque. This property mystified ancient alchemists, because, generally, substances become liquid when heated. The protein in egg white albumin helps baked goods hold their structure, and the same egg white albumin is also used for purification, as it tends to trap and store impurities. In addition, albumin is used to treat people with certain types of poisoning, since it binds to the toxin.

In the preparation of bakery products, in practice, many additives are used to improve the properties of the dough: eggs, powdered soy, milk, etc. Jacquay and coll.[114] have investigated the modifications, such as desirability and strength, in meatloaf as caused by different hamburger binders: egg, powder skim milk and powdered soymilk. Bovine blood plasma is a useful source of high-quality protein and exhibits many useful properties. Blood plasma, used in bakery products instead of egg albumin, has good foaming and leavening properties. Adding plasma (no more of 2%) to bread flour encourages a considerable higher loaf volume and also increases the protein quality. Thus the bread contains about 15% more protein and around 75% more lysine. Spray-dried plasma can be used as an egg substitute. Plasma levels greater than 2% darkened the crust and make the texture of bread more open and coarse. Many researches[115] found that in cake making about 30% of whole egg could be replaced with blood plasma. In those studies, full replacement with blood plasma did not quite produce the same volume, crumb structure and profile as egg white.

Raeker et al[116] have investigated the cake-baking properties of egg white and blood plasma. They concluded that egg white produced slightly larger cake volume, a significantly more crowned profile, and a finer crumb structure than did blood plasma. Among the blood plasma proteins, fibrinogen produced the smallest cake volume. Albumin, the protein in blood plasma, had cake-baking properties inferior to those of whole blood plasma.

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114. Jacquay A., C. Beaver, S. Fisher (2011), ibid., Research Project-Fall (www.cfs.purdue.edu/fn/fn453/).

115. Brooks J., P. W. Ratcliff (1959), "Dried bovine plasma. I. Storage of spray-dried plasma and the freeze-concentration of liquid plasma", *Journal of the Science of Food and Agriculture*, 10 (9): 486-495; Khan M. N., L. W. Rooney, C. W. Dill (1979), "Baking properties of plasma protein isolate", *Journal of Food Science*, 44 (1): 274-76; Johnson L. A., E. F. Havel, R. C. Hoseney (1979), "Bovine plasma as a replacement for egg in cakes", *Cereal Chemistry*, 56: 339-41; Lee C. C., L. A. Johnson, J. A. Love, S. Johnson (1991), "Effects of processing and usage level on performance of bovine plasma as an egg white substitute in cakes", *Cereal Chemistry*, 68 (1): 100-104.

116. Raeker M. Ö., L. A. Johnson (1995), "Cake-Baking (High-Ratio white Layer) Properties of Egg white, Bovine Blood Plasma, and their Protein Fractions", *Cereal Chemistry*, 72 (3): 299-303.

Emulsifier

An emulsifier or emulsifier agent is a substance that will hold another substance suspended so that the solution is stable. Emulsifiers are utilized in emulsified meat to bind meat proteins, fat and water in a stable emulsion. In the food industry, among the emulsifiers, casein and its salt derivatives are widely used. But, because of its high processing costs, it is expensive. Proteins are also common emulsifying agents as they occur naturally and are generally widely available, non-toxic and inexpensive.[117] According to some research, protein isolate produced from food industry by-products may have potential as an emulsifying agent in the food industry. Blood proteins have been found to have emulsifying properties that are comparable, and often, superior to those of casein and can therefore replace casein as an emulsifying agent. Silva et al. used two methods for extraction of globin from bovine blood, in order to test it in terms of efficiency, protein recovery and solvent residues.[118]

The evaluation of the capacity of blood proteins in either forming or stabilizing emulsions is highly important from an industrial point of view since the manufacture of several foods, such as mayonnaise, pates and sausages involves an emulsification process. Moreover, the incorporation of proteins in food products may increase their nutritional value. On the other hand, considering that the action of proteins as emulsifiers is complex and depends on different factors (protein concentration, oil type, velocity and length of mixture, among others), it is important to study the behavior of proteins under different conditions.[119] Bizzotto et al.[120] have evaluated the emulsifying properties of a blood protein. They studied the effect of the pH and of the tryptic hydrolysis on the emulsifying properties of bovine globin and determined the emulsifying capacity (EC), the emulsifying activity index (EAI), and the emulsion stability (ES) at pH varying from 3.0 to 8.0 and employing hydrolysis times from 5 to 60 minutes. They obtained the highest values for EC and ES at pH 5.0 and 6.0, respectively, corresponding to the range of high protein solubility. The best results for the emulsifying properties of bovine globin, extracted by the acidified acetone method, was observed in the acid region (pH from 3.0 to 6.0), where this protein is highly soluble.

Finally, blood proteins (hemoglobin), used as emulsifiers, have the further advantage of providing a good source of heme iron.[121] The success of blood-fortified foods in addressing iron

117. Matsumura Y., K. Matsumiya (2012), Chapter 5, *Proteins–Peptides as Emulsifying Agents*, in S. Navam, Hettiarachchy, K. Sato, M. R. Marshall, A. Kannan, *Food Proteins and Peptides Chemistry, Functionality, Interactions, and Commercialization*, CRC Press, Florida (USA), pp. 125–150.

118. Silva J. G., H. A. Morais, M. P. C. Silvestre (2003), "Comparative study of the functional properties of bovine globin isolates and sodium caseinate", *Food Research International*, 36 (1): 73-80.

119. Tybor P. T., C. W. Dill, W. A. Landmann (1973), "Effect of descolorization and lactose incorporation on the emulsification capacity of spray-dried blood protein concentrates", *Journal of Food Science*, 38 (6): 4-6; Crenwelge D. D., C.W. Dill, P.T. Tybor, W. A. Landmann (1974), "A comparison of the emulsification capacities of some protein concentrates", *Journal of Food Science*, 39 (910): 175-177; Gauthier S. F., P. Paquin, Y. Pouliot, S. Turgeon (1993), "Surface activity and related functional properties of peptides obtained from whey proteins", *Journal of Dairy Science*, 76 (1): 321-328.

120. Schaper Bizzotto C., M. Capobiango, M. Pinto Coelho Silvestre (2005), "Evaluation of Functional Properties of a Blood Protein", *Pakistan Journal of Nutrition*, 4 (1): 11-16.

121. As bovine blood has the largest amount of heme iron than any animal source (see: Kikafunda J. K., P. Sserumaga (2005), ibid., *African journal of food agriculture nutrition and development*, 5 (1): 1-18) and is readily available, it has therefore been suggested for the fortification of staple foods.

deficiency has been reported by several researchers.[122] Compared to egg and casein, that are potent allergens that are among the "big eight" allergens[123] covered under the "Food Allergen Labeling and Consumer Protection Act" (FALCPA) law[124], blood proteins present no problem.

Fat replacers

Fat, besides its nutritional function, plays an important role in the diet as a source of essential fatty acids and energy; as an enhancer of tenderness, palatability and juiciness; and as a binder of processed meats. As empirical data[125] has shown a correlation between dietary fat and cardio-vascular disease and some types of cancers, consumers have reacted by reducing dietary fat in their food.[126] In response to this trend, the food industry has developed an assortment of low-fat meat products. But given the sensory role played by fats, replacing their presence in food products is a difficult task. Many researchers[127] have shown blood proteins

122. According to Nissenson A. R., C. Charytan (2003) (in "Management of Comorbidities in Kidney Disease in The 21St Century: Anemia and Bone Disease", Kidney International, 64: S64–S71), "although oral non-heme iron is infrequently sufficient to maintain iron stores in hemodialysis patients, recent studies suggest that heme-iron may be more useful in this regard. Heme-iron is absorbed to a greater extent than non-heme iron, and is better tolerated. Small studies have shown that when heme-iron is administered, less parenteral iron and lower doses of erythropoietin (EPO) are needed to maintain target haemoglobin"; See also: Hurrell R. F. (1997), "Preventing iron deficiency through food fortification", Nutrition Reviews, 55 (6): 210-22; Nissenson A. R., J. S. Berns, P. Sakiewicz, S. Ghaddar, G. M. Moore, R. B. Schleicher, P. A. Seligman (2003), "Clinical evaluation of heme iron polypeptide: sustaining a response to rHuEPO in hemodialysis patients", *American Journal of Kidney Diseases*, 42 (2): 325-330; Seligman P. A., G. M. Moore, R. B. Schleicher (2000), "Clinical studies of hip: An oral heme-iron product", *Nutrition Research*, 20 (9): 1279-1286

123. A group of the eight major allergenic foods is often referred to as the Big-81 and comprises milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybean. These foods account for about 90% of all food allergies in the USA.

124. The "Food Allergen Labeling and Consumer Protection Act" of 2004 (FALCPA) (Public Law 108-282) was enacted in August 2004, and addresses, among other issues, the labeling of foods that contain certain food allergens.

125. See among others: Takashi Sugimura (1997), "Food and cancer prevention", *Cancer Letters*, 114 (1–2): 3–5; Greenwalda P., C. K. Cliffordb, J. A. Milner (2001), "Diet and cancer prevention", *European Journal of Cancer*, 37 (8): 948-965; D'Avanzo B., E. Negri, A. Gramenzi, S. Franceschi, F. Parazzini, P. Boyle, C. La Vecchia (1991), "Fats in seasoning and breast cancer risk: an Italian case-control study", *European Journal of Cancer and Clinical Oncology*, 27 (4): 420-423; Ferguson L. R. (2010), "Meat and cancer", *Meat Science*, vol. 84 (2): 308-313; Hooper L., C. D. Summerbell, J. P. T. Higgins, R. L. Thompson, N. E. Capps, G. Davey Smith, R. A. Riemersma, S. Ebrahim (2001), "Dietary fat intake and prevention of cardiovascular disease: systematic review", *BMJ*, 322 (7289): 757–763.

126. Colmenero F. J. (1996), "Technologies for developing low-fat meat products", *Trends in Food Science & Technology*, 7 (2): 41-48.

127. Read more: Totosaus A., R. H. Alfaro-Rodriguez, M. L. Pérez-Chabela (2004), "Fat and sodium chloride reduction in sausages using κ-carrageenan and other salts", *International Journal of Food Sciences and Nutrition*, 55 (5): 371-380; Tokusoglu O., M. K. Unal (2003), "Fat Replacers in Meat Products", *Pakistan Journal of Nutrition*, 2: 196-203; Joly G., B. Anderstein (2009), *Starches, Ingredients in Meat Products*, Springer-Verlag, New York, pp 25-55; Viana F. R., V. D. M. Silva, F. M. Delvivo, C. S. Bizzotto, M. P. C. Silvestre (2005), "Quality of ham pate containing bovine globin and plasma as fat replacers", *Meat Science*, 70 (1): 153-160; Viana F. R., C. S. Bizzotto, D. R. Dias, A. L. Oliveira, M. P. C. Silvestre (2004), "Bovine Blood Constituents as Fat Replacers in Ham Pâté", *Food Technology and*

can replaced fat in meat products, while at the same time having the advantage of reducing costs and the caloric content of food.

Imitation seafood

Surimi (from the Japanese[128] "ground meat") is a fish-based food product that has been pulverized to a thick paste. When cooked it has the appearance of a rubbery food item. In general, surimi is sold as imitation crab or mock crab in USA and as seafood sticks, crab sticks, or fish sticks in Europe. Imitation crabmeat is a seafood product made by blending processed fish with various texturizing ingredients, flavorants, and colorants resulting in a gel.[129] This gel can be shaped and cut into thin strips which, when rolled together, mimic the texture of real crabmeat.

Depending on the desired texture and flavor of the surimi product, the gelatinous paste is mixed with differing proportions of additives such as egg white, starch, salt, vegetable oil, humectants, sorbitol, sugar, soy protein, seasonings, and enhancers such as transglutaminases and monosodium glutamate. The coloring for imitation crabmeat is made using compounds like carmine, caramel, paprika, and annato extract.

Stabilizers

Stabilizers are substances or chemicals that allow food ingredients, which do not mix well, to remain in a homogenous state after blending. Transglutaminase is widely used in seafood, surimi products, meat products, noodles/pasta, dairy products, baked goods, whipped toppings[130] and so on. It has great potential to improve the firmness, elasticity, viscosity, heat stability, and water-holding capacity of prepared foods through mild enzyme reaction.[131]

b) Feed (blood meal)

Blood can be collected during the slaughter of various livestock species (cattle, pigs, chickens, etc.) and is usually dried and made into blood meal so that it can be handled and incorporated into animal rations and can be used as a stabilizer for fat in bone meal. The yield of blood meal[132] from whole blood is approximately 20%.

Biotechnology, 42 (1): 5–10; Carballo J., G. Barreto, F. J. Colmenero (1995), "Starch and Egg White lnfluence on Properties of Bologna Sausage as Related to Fat Content", *Journal of Food Science*, 60 (4): 673–677; Abiola S. S., S. W. Adegbaju (2001), "Effect of substituting pork backfat with rind on quality characteristics of pork sausage", *Meat Science*, 58 (4): 409-12.

128. The Japanese have been using surimi-based products for about a thousand years. Traditionally called "kamaboko", the first recorded surimi manufacturing procedure was found in a Japanese cookbook written in 1528.

129. Lean meat from fish (or land animals) is first separated or minced. Then it is rinsed many times to eliminate undesirable odours and pulverized to form a gelatinous paste.

130. Whipped toppings are concoctions of sugar, stabilizers, and some substance approximating cream.

131. As a review see: Kuraishia C., K. Yamazakia, Y. Susa (2001), "Transglutaminase: its Utilization in the Food Industry", *Food Reviews International*, 17 (2): 221-246.

132. Blood run through a decanter to separate the coagulated blood into pre-dewatered blood meal and blood water, which is released during coagulation. The blood meal is then cooked with stirring to avoid clumping. Sometimes it is added lime (70% calcium oxide), at the 0.5-1.5% level, to increase storage life and to decrease the odour released during drying. Blood mixed with lime has a rubbery

Blood meal contains mostly protein and is used as a source of protein to supplement diets based on forages, plant by-products and cereal grains. It has been shown to be a satisfactory replacement for other supplemental protein sources in various animal productions including dairy cattle, beef cattle, sheep, pigs, poultry, various fish species, and silkworms. It is also a good source of most of the trace minerals. Blood meal is rich source of lysine but is deficient in tryptophan and isoleucine.

Blood meal is often hydroscopic and needs to be dried to less than 10-12% moisture and stored in a dry place. There are different ways to prepare blood meal: the drying method used (solar, oven, drum, flash, spray drying, etc.) has different effects on the digestibility of the blood meal proteins. There is an inverse relationship between the amount of heat applied and protein digestibility: lysine content decreases when the amount of heat increases.[133]

It is worth noting, however, that the advent of "mad cow" disease (Bovine Spongiform Encephalopathy or BSE) raised international concern about the safety of feeding rendered cattle to cattle. Since the discovery of mad cow disease in the USA, EN and other countries, governments have taken action to restrict certain parts of cattle that can be fed back to cattle. The rise in animal factories over the last 50 years has led to a system that is out of control.

However, most animals are still allowed to eat animal by-products from their own species. Pig carcasses can be rendered and fed back to pigs, cattle can be fed cow blood and some other cow parts, chicken carcasses can be rendered and fed back to chickens, and so on. Also, cosmetics and dietary supplements cannot be manufactured using potentially infectious cow parts. In recent years, some supplements have claimed to include cow brains.

c) Fertilizers

Blood is useful for modifying the pH of the soil and to add nitrogen. It is also useful to improve the soil texture. Its disadvantage is the possibility of attracting rats and vermin when

consistency and can be stored at 20° C for 24 hours without spoilage (it contains 80-85% moisture). In order to remove moisture, the dark-brown cooked product is pressed and dried to the desired moisture level. The dried product is then ground and used as feed (80% protein). Spray-dried blood can also be used as an adhesive, in asphalt emulsions, in insecticides, in ceramics, and as a substitute for egg albumin when colour is not important. The dried product is often heated to 100° C for 30 minutes, cooled, and stored in airtight containers.

133. See: Johns D. C., C. K. Low, J. R. Sedcole, M. P. Gurnsey, K. A. C. James (1987), "Comparison of several in vivo digestibility procedures to determine lysine digestibility in poultry diets containing heat treated meat and bone meals", *British Poultry Science*, 28 (3): 397–406; Wang X., C. M. Parsons (1998), "Bioavailability of the Digestible Lysine and Total Sulfur Amino Acids in Meat and Bone Meals Varying in Protein Quality", *Poultry Science*, 77 (0): 1003–1009; Batterham E. S., R. E. Darnell, L. S. Herbert, E. J. Major (1986), "Effect of pressure and temperature on the availability of lysine in meat and bone meals as determined by slope-ratio assays with growing pigs, rats and chicks and by chemical techniques", *British Journal of Nutrition*, 55 (2): 441–453; Batterham E. S., R. F. Lowe, R. E. Darnell (1986), "Availability of lysine in meat meal, meat and bone meal and blood meal as determined by the slope-ratio assay with growing pigs, rats and chicks and by chemical techniques", *British Journal of Nutrition*, 55 (2): 427-440; Lewis A. J., D. H. Baker (1995), *Bioavailability of D-Amino acids and DL-Hydroxy Methionine*, in C. B. Ammerman, D. H. Baker, and A. J. Lewis (Edtrs), *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*, Academic Press, London (UK)-San Diego (USA), pp. 67-81; Parsons C. M., F. Castanon, Y. Han (1997), "Protein and amino acid quality of meat and bone meal", *Poultry Science*, 76 (2): 361–368.

spread on the soil. The dried product is used as fertilizer (12% nitrogen, 0.22% phosphorus, and trace elements). It is usually mixed with super phosphates to make a compounded fertilizer.

Gagnon and Berrouard[134] evaluated the potential of different organic wastes from the agri-food industry for growing greenhouse tomato transplants. The organic materials were thoroughly mixed with a peat-compost growing medium prior to transplanting. Meal from blood, feathers, meat, crab shells, fish, cottonseed and whey by-products produced the best growth, significantly increasing the shoot dry weight by 57-83% compared with non-fertilized plants.

Ciavattaa et al.[135] had incubated blood meal in the soil for one year, following the evolution of the organic matter. The results showed that only a part (about 75%) of the organic carbon (C) and of the organic N mineralized and that the remaining C was transformed into humified compounds. The availability of Fe increased during the incubation, probably due to the progressive degradation of the prosthetic group and the successive chelation of the Fe from the humic substances.

In a laboratory incubation Hartz and Johnstone[136] compared the rate of net nitrogen mineralization (N_{min}) from seabird guano, hydrolyzed fish powder, feather meal and blood meal. Across temperatures, all fertilizers had equivalent N_{min} after 15 days, with blood meal having a slight advantage after 2 months.

According to Shepherd,[137] blood meal is sometimes not recommended for organic gardening because it can damage young tender plants in warm moist conditions.

d) Scientific use or research

Animals can produce useful medical substances in their blood, like vaccines, hormones, and antibodies, which are important for basic research, diagnostic tests, and medical treatments. Human biology is very much like that of many other animals. That is why results from animal experiments apply to people. Most laboratory animals have the same set of organs (heart, lungs, liver, and so on) that work in the same way as they do in humans.[138]

e) Laboratory uses

Serum

It is widely used in cell culture, microbiology procedures and diagnostic kits. Blood from donor animals continues to play an important role as a nutritional supplement in microbiological culture media. Many isolated blood components are used in chemical analysis or as nutritive supplements. Blood plasma is used as a diluent for boar and bull semen.

134. Gagnon B., S. Berrouard (1994), "Effects of several organic fertilizers on growth of greenhouse tomato transplants", *Canadian Journal of Plant Science*, 74: 167-168.
135. Ciavatta C., M. Govia, L. Sittia, C. Gessa (1997), "Influence of blood meal organic fertilizer on soil organic matter: A laboratory study", *Journal of Plant Nutrition*, 20 (11): 1573-1591.
136. Hartz T. K., P. R. Johnstone (2006), "Nitrogen Availability from high-nitrogen-containing Organic Fertilizers", *Hort Technology*, 16 (1): 39-42.
137. Shepherd A. (2003), *How to make soil and save Earth*, CAT Publications, Machynlleth, Powys, UK.
138. To know more about see: Giridharan N. V., Vijay Kumar, Vasantha Muthuswamy (2000), *Use of Animals in Scientific Research*, Indian Council of Medical Research, Ministry of Health & Family Welfare, New Delhi.

Factors

The 13 blood clotting factors that control clot are: Factor I = Fibrinogen, Factor II = Prothrombin, Factor III = Tissue factor, Factor IV = Calcium, Factor V = Labile factor, Factor VI – (Does not exist as it was named initially but later on discovered not to play a part in blood coagulation), Factor VII = Stable factor, Factor VIII = Antihemophilic factor A, Factor IX = Antihemophilic factor B or Christmas factor, Factor X = Stuart Prower factor, Factor XI = Antihemophilic factor C, Factor XII = Hageman factor, and Factor XIII = Fibrin stabilizing factor.

Coagulation factors (also known as Blood Clotting Factors, Clotting Factors, or Factor Assays) or by the individual factor number (Factor I, Factor II, etc.) or name (Fibrinogen, Prothrombin, etc.). Coagulation factor tests may be carried out in the presence of excessive bleeding or hematomas or a prolonged Prothrombin Time (PT) or Partial Thromboplastin Time (PTT). These tests are used as screening tools to determine whether there is a coagulation problem. Factor activity may be measured in case of appropriate signals of having an acquired condition that is causing bleeding, such as vitamin K deficiency or liver disease.

Factor testing may be done when bleeding episodes begin early in life and an inherited coagulation factor deficiency is suspected. In this case, other family members may also be tested to help confirm the person's diagnosis.

Blood albumin

The albumin blood test measures the amount of albumin in the blood serum.[139] It can be used to evaluate how the liver and kidneys are functioning.[140] Low levels (hypoalbuminemia) can indicate liver damage, while high levels usually reflect dehydration. Low albumin levels can also be seen in inflammation, shock, malnutrition, excess excretion by the kidneys (as in nephrotic syndrome), excess loss in bowel (protein-losing enteropathy), burns (plasma loss in the absence of skin barrier), redistribution (hemodilution as in pregnancy), and mutation causing analbuminemia (very rare), etc.[141]

139. Tietz N. W. (Edt.) (1995), *Clinical Guide to Laboratory Tests*, 3rd ed. W. B. Saunders, Philadelphia, PA; Nicoll D., S. J. McPhee, M. Pignone, Chou T. M. Detmer (edts) (2001), *Pocket Guide to Diagnostic Tests*, 3rd. McGraw-Hill, New York; Ballmer P. E., M. A. McNurlan, E. Milne, S. D. Heys, V. Buchan, A. G. Calder, P. J. Garlick (1990), "Measurement of albumin synthesis in humans: a new approach employing stable isotopes", *American Journal of Physiology*, 259 (6 Pt 1): E797–E803.

140. Berk P. D., K. M. Korenblat (2007), *Approach to the patient with jaundice or abnormal liver test results* (in: L. Goldman and D. Ausiello, Edts. Cecil Medicine, Saunders Elsevier, Philadelphia, Pa; Doumas B. T., W. A. Watson, H. G. (1971), "Biggs Albumin standards and the measurement of serum albumin with bromcresol green", *Clinica Chimica Acta*,31 (1): 87–96.

141. Among others: Fearon K. C., J. S. Falconer, C. Slater, D. C. McMillan, J. A. Ross, T. Preston (1998), "Albumin synthesis rates are not decreased in hypoalbuminemic cachectic cancer patients with an ongoing acute-phase protein response", *Annals of Surgery*, 227 (2): 249–254; Ballmer P. E., A. F. Ochsenbein, S. Schütz-Hofmann (1994), "Transcapillary escape rate of albumin positively correlates with plasma albumin concentration in acute but not in chronic inflammatory disease", *Metabolism*, 43 (6): 697–705; Fleck A., G. Raines, F. Hawker, J. Trotter, P. I. Wallace, I. M. Ledingham, K. C. Calman (1985), "Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury", *Lancet*, 1 (8432): 781–784.

Fibrinogen

It is a protein produced by the liver that helps stop bleeding by helping blood clots to form. A blood test can be done to know how much fibrinogen there is in the blood. Normal levels are about 1.5-3 g/l, depending on the method used. In typical circumstances, fibrinogen is measured in citrated plasma samples in the laboratory, however the analysis of whole blood samples by use of thrombelastometry is also possible. Higher levels (>3.43 g/l) are often associated with cardiovascular disease. Fibrinogen levels may elevate in any form of inflammation, as it is an acute-phase protein.[142] In pregnancy, fibrinogen levels increase to an average of 4.5 g/l, compared to an average of 3 g/l in non-pregnant women.

The acute phase response is a physiologic reaction to infection or trauma. The increase in plasma acute phase proteins provides an objective measure of inflammation in response to disease and therapeutic intervention.[143] Research in human and veterinary medicine has focused on acute phase proteins as an early marker in inflammation that can indicate the time and degree of symptom. The two most common acute phase proteins evaluated in horses are fibrinogen and serum amyloid A (SAA).[144] SAA increases rapidly (more than 100 fold) after tissue injury but has a short half-life. Fibrinogen increases more slowly (about 24-72 hours) after the induction of inflammation but to a lower degree (among 1-10 fold).

For this reason it is used in veterinary medicine as an inflammatory marker[145]. Fibrinogen and Haptoglobin are good markers of chronic inflammatory disease and very useful for monitoring out-patients in which samples are taken less frequently.[146] In horses, a Fibrinogen level above the normal range of 1.0-4.0 g/l suggests some degree of systemic inflammatory response. Haptoglobin levels correlate with the clinical status of disease in cases of musculoskeletal, respiratory and digestive pathologies. The presence of an inflammation process was better confirmed by Fibrinogen presence in cases of cutaneous and musculoskeletal pathologies. When considering a wide range of diseased horses, it appeared that Haptoglobin has a better sensi-

142. See: Hultén C., U. Grönlund, J. Hirvonen, R. -M. Tulamo, M. M. Suominen, G. Marhaug, M. Forsberg (2002), "Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and α2-globulins during induced noninfectious arthritis in the horse", *Equine Veterinary Journal*, 34 (7): 699–704.

143. Crisman M., W. Scarratt, K. Zimmerman (2008), "Blood proteins and inflammation in the horse", *Veterinary Clinics of North America: Equine Practice*, 24 (2): 285–297.

144. Hultén C., et al., "Dynamics in serum of the inflammatory markers serum amyloid A(SAA), haptoglobin, fibrinogen and a2-globulins during induced noninfectious arthritis in the horse", ibid., pp. 699-704; Jacobsen S., H. Thomsen, S. Nanni (2006), "Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease", *American Journal of Veterinary Research*, 67 (10): 1738-1742.

145. Allen B. V., S. E. Kold (1988), "Fibrinogen response to surgical tissue trauma in the horse", *Equine Veterinary Journal*, 20 (6): 441-443; Larsson A., J. Bjork, C. Lundberg (1997), "Nephelometric determination of rat fibrinogen as a marker of inflammatory response", *Veterinary Immunology and Immunopathology*, 59 (1): 163-169.

146. Paolaggi J. B., D. Chaouat, D. Barres, H. Hoffman, L. Auquier (1982), "Comparative variations of the sedimentation rate, haptoglobin and orosomucoid in rhizomelic pseudopolyarthritis and temporal arteritis. Attempt at a definition of biological parameters for monitoring these diseases", *Revue du rhumatisme et des maladies ostéo-articulaires*, 49 (6): 413-419.

tivity than Fibrinogen.[147] Mair and Linnenkohl[148] hypothesized that patients presenting for colic associated with inflammatory conditions (peritonitis, colitis, inflammatory bowel disease, strangulating surgical lesions) would display significantly higher concentrations of Fibrinogen and SAA compared to patients that presented for non-specific medical colic.

According to Borges et al.[149] measurement of plasma iron concentration better reflected acute inflammation than did fibrinogen concentration.

Globulins

The two main proteins in the blood are albumin and globulin both produced by the liver. Others are made by the immune system. Globulin carries essential metals through the bloodstream and carries them to the various parts of the body and helps to fight infections. Globulin proteins include enzymes, antibodies and more than 500 other proteins. All plasma proteins except albumin and prealbumin are globulins. The plasma globulins are separated into five fractions by serum protein electrophoresis (SPE).[150] In order of decreasing electrophoretic mobility these fractions are the alpha1-, alpha 2-, beta1-, and beta 2-globulins, and the gamma globulins.

Sphingomyelin

It is one of the major lipids of the plasma membranes of mammalian cells. It is a sphingolipid found in animal cell membranes, especially in the membranous myelin sheath that surrounds nerve cell axons. Its function remained unclear until recently, when it was found to have a role in signal transduction.[151] Analysis of sphingomyelin can be carried out by means of chromatography[152] or by mass spectrometry.[153]

147. Oukacha F., P. Bertrand, J. Bustin, M. Humblet, H. Amory, J. Godeau (2005), *Relationship between haptoglobin, fibrinogen and clinical status in horses*, 5th International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland, March 14-15, Poster 13, p. 62.

148. Mair T., W. Linnenkohl (2011), *The use of serum amyloid A (SAA) and fibrinogen as disease markers in equine colic*, Bell Equine Veterinary Clinic, Mereworth, Kent, UK (e-mail: wlinnenkohl@gmail.com.).

149. Borges A. S., T. J. Divers, T. Stokol, O. Hussni Mohammed (2007), "Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses", *Journal of Veterinary Internal Medicine*, 21 (3): 489-494.

150. For more details see: Tiselius A. (1937), "Electrophoresis of serum globulin. Electrophoretic analysis of normal and immune sera", *Biochemical Journal*, 31 (9): 1464–1477; Peacock A. C., S. L. Bunting, K. G. Queen (1965), "Serum protein electrophoresis in acrylamide gel: patterns from normal human subjects", *Science*, 147 (3664): 1451-1453; Vanderschaeghe D., E. Debruyne, H. Van Vlierberghe, N. Callewaert, J. Delanghe (2009), "Analysis of gamma-globulin mobility on routine clinical CE equipment: exploring its molecular basis and potential clinical utility", *Electrophoresis*, 30 (15): 2617-23; El-Zarkouny S. Z., M. M. Shaaban, J. S. Stevenson (2011), "Blood metabolites and hormone-based programmed breeding treatments in anovular lactating dairy cows", *Journal of Dairy Science*, 94 (12): 6001-10.

151. Kolesnick R. (1994), "Signal transduction through the sphingomyelin pathway", *Molecular and chemical neuropathology*, 21 (2-3): 287-97.

152. Ramstedt B., P. Leppimäki , M. Axberg, J. P. Slotte (1999), "Analysis of natural and synthetic sphingomyelins using high-performance thin-layer chromatography", *European Journal of Biochemistry*, 266 (3): 997-1002; Lee S., Youn-Sun Lee, Kyeong-Mi Choi, Kwang-Sik Yoo, Dong-Mi Sin, Wonkyun Kim, Yong-Moon Lee, Jin-Tae Hong, Yeo-Pyo Yun, Hwan-Soo Yoo (2012), "Quantitative Analysis of Sphingomyelin by High-Performance Liquid Chromatography after Enzymatic Hydrolysis", *Evidence-Based Complementary and Alternative Medicine*, Article ID 396218: 1-9.

153. Sullards M. C. (2000), "Analysis of sphingomyelin, glucosylceramide, ceramide, sphingosine, and

Catalase

It is an extraordinary enzyme. Catalase is ubiquitous and is found in animal[154] and in plant cells. It catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen.[155] This enzyme is used in laboratories as a tool for learning the effect of enzymes upon reaction rates. The test (catalase test) is one of the main tests used by microbiologists to identify species of bacteria.[156]

Agglutination test

It is a blood test used to identify unknown antigens. Blood with the unknown antigen is mixed with a known antibody; whether or not agglutination occurs helps to identify the antigen (qualitative agglutination test). Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, sample serial dilutions are tested for antibody and then a fixed number of red blood cells, bacteria, or other such particulate antigens are added to determine the maximum dilution for agglutination. Agglutination testing is also used in tissue matching, blood grouping, and diagnosis of infections.[157]

Diagnostic microbiology

Microbes such as bacteria, protozoans, and fungi play a role in many disease processes. Since the 1960s, numerous ingenious innovations have been introduced and used in clinical microbiology laboratories. Miniaturization, plastic disposables, and commercial medium production are the norm in present-day microbiology laboratories. Mechanization of analytical microbiological procedures have given way to automation. Robotization, now available for the routines of chemistry, hematology, endocrinology, some serology, and urinalysis, may eventually find applications in some, if not many, aspects of clinical microbiology.[158]

Infectious diseases are often diagnosed following cultures of samples isolated from the infection site. Many bacteria, fungi, parasites and viruses can be grown in a lab when appropriate conditions are used. Precise characteristics of the growing cultures can be used to identify specific microbe. Diagnostic cultures are commonly used to identify infectious

sphingosine 1-phosphate by tandem mass spectrometry", *Methods in Enzymology*, 312: 32-45; Shaner R. L., J. C. Allegood, H. Park, E. Wang, S. Kelly, C. A. Haynes, M. C. Sullards, A. H. Merrill, Jr. (2009), "Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers", *The Journal of Lipid Research*, 50 (8): 1692–1707.

154. Murthy M. R., T. J. Reid, A. Sicignano, N. Tanaka, M. G. Rossmann (1981), "Structure of beef liver catalase", *Journal of Molecular Biology*, 152 (2): 465–499.

155. Chelikani P., I. Fita, P. C. Loewen (2004), "Diversity of structures and properties among catalases", *Cellular and Molecular Life Sciences*, 61 (2): 192–208.

156. Gagnon M., W. Hunting, W. B. Esselen (1959), "A new method for catalase determination", *Analitical Chemistry*, 31 (1): 144–146; Duke P. B., J. D. Jarvis (1972), "The catalase test—a cautionary tale", *The Journal of Medical Laboratory Technology*, 29 (2): 203–204.

157. A particular use of this test is reported by: Verloo D., E. Magnus, P. Büscher (2001), "General expression of RoTat 1.2 variable antigen type in Trypanosoma evansi isolates from different origin", *Veterinary Parasitology*, 97 (3): 183-189.

158. See: Isenberg H. D. (2003), "Clinical Microbiology: Past, Present, and Future", *Journal of Clinical Microbiolology*, 41 (3): 917-918.

microbes from samples isolated from urine, stool, genital tract, throat, skin, blood, and the spinal cord.[159,160]

f) Pharmaceutical uses

Animals and their parts have been used as medicinal resources for the treatment and relief of a myriad of illnesses and diseases in practically every human culture. Pliny tells us that the blood of animals (human blood as well) was administered in his time for curative purposes; so likewise, today the blood of the ox is in medicinal vogue in certain parts of the Eastern Hemisphere.

Blood is an excellent source of raw materials for making pharmaceuticals and to carry out analysis of the following: immunoglobulins, thrombin (blood clotting), factors, sutures, fibrinogen, fibrinolysin, fibrin products, serotonin, kalikrenis, plasminogen, plasma extenders, and transfusions. Chemically the blood of animals contains a considerable quantity of iron, in addition to albumin, fibrin, hydrogen, some traces of prussic acid, and some empyreumatic oil. The serum, or thin part of the blood, includes sulphur. Many blood products are made from the plasma component of blood. Plasma contains a large number of proteins, each of which performs a different role within the blood.

Immunoglobulins are one of the most important mechanisms the body has to protect itself against infections. If the patient has depleted quantities in his or her blood, the use of immunoglobulin concentrates enables replacement. These blood products are widely used for immune deficiencies, such as for cancer patients undergoing chemotherapy.

The products most commonly isolated from plasma and their uses are overviewed in the following:[161]

• Whole Blood	Transfusions or broken down into components;
• Red Cells	Anemia and replacement of traumatic or surgical blood loss;
• Platelets	Hemorrhage;
• Fresh Frozen Plasma	Bleeding problems after trauma or liver transplants;
• Albumin	Blood volume replacement i.e. after accident;

159. Isenberg H. D., A. Reichler, D. Wiseman (1971), "Prototype of a fully automated device for determination of bacterial antibiotic susceptibility in the clinical laboratory", *Applied Microbiology*, 22 (6): 980-986.

160. To be informed of the latest developments in clinical microbiology and the diagnosis and treatment of infectious diseases, among many, see: Mahon C. R., D. C. Lehman, G. Manuselis, Jr. (2007), *Textbook of Diagnostic Microbiology*, Volume 355, Elsevier Science - Health Science Division, p.1211; *Diagnostic Microbiology and Infectious Disease 2012*, Elsevier, Edt. Ronald N. Jones, 74 (1): 323-434; Forbes B. A., D. F. Sahm, A. S. Weissfeld (2007), *Bailey & Scott's Diagnostic Microbiology*, Elsevier Science.

161. The 2009 Report on Blood and Blood Derivatives and Fractions excluding those used for passive immunization and diagnostics: World Market Segmentation by City. Parker, Philip M.//City Segmentation Reports; 1/3/2010, pN. PAG.

• Immunoglobuline	Immune deficiencies i.e. often used with patients with Cancer receiving chemotherapy;
• Factor VIII	Hemophilia A;
• Factor VIII/vWF (von Willebrand complex)	von Willebrand Disease;
• Factor IX	Hemophilia B;
• Factor XI	Hemophilia A
• Alpha-1-proteinaise inhibitor	Acquired and congenital emphysema. Cystic fibrosis;
• C-1-esterase inhibitor	Hereditary angioedema. Heart attacks;
• Protein C	Purpura fulminans. Couramin induced skin necrosis;
• Prothrombin Complex Concentr.	Bleeding disorders. Hemophilia B;
• Anticoagulation Complex	Bleeding disorders;
• Antithrombin III	Deep Vein Thrombosis. Pulmonary embolus;
• Fibrinogen	Stopping of bleeding or hemorrhaging in surgery;
• Thrombin	Stopping of bleeding or hemorrhaging in surgery.

Brantl and coll.,[162] Nyberg and coll.,[163] and Liebmann and coll.[164] have studied the properties of opioid peptides derived from hemoglobin.

Immunoglobulins

Glycoprotein molecules that are produced by plasma cells in response to an immunogen function as antibodies, are called immunoglobulins. These immunoglobulins are synthesized by B-lymphocytes. Their derivative plasma cells are found in the blood serum and in other body fluids and tissues, including the urine, spinal fluid, lymph nodes and spleen. Immunoglobulin molecules consist of two kinds of polypeptide chains: heavy chains (H-chains) and light chains (L-chains). There are five antigenically different kinds of H-chains, designated γ, μ, α, δ and ε, based on differences in the amino acid sequences in the constant region of the H-chains. This difference is the basis for the classification of immunoglobulins. Classes vary in their chemical structure and in the number of antigen-binding sites. The classes of immunoglobulins can divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains.

162. Brantl V., C. Gramsch, F. Lottspeich, R. Mertz, K. H. Jaeger, A. Herz (1986), "Novel opioid peptides derived from hemoglobin: hemorphins", *European Journal of Pharmacology*, 125 (2): 309–10.
163. Nyberg F., K. Sanderson, E. L. Glämsta (1997), "The hemorphins: a new class of opioid peptides derived from the blood protein haemoglobin", *Biopolymers*, 43 (2): 147–56.
164. Liebmann C., U. Schrader, V. Brantl (1989), "Opioid receptor affinities of the blood-derived tetrapeptides hemorphin and cytochrophin", *European Journal of Pharmacology*, 166 (3): 523–6.

The five classes of immunoglobulins (Ig) are: IgA,[165] IgD,[166] IgE,[167] IgG,[168] IgM.[169] Only IgG, IgM and IgA and are present in all species of domestic animals.[170]

Blood clotting or blood clot

It consists of a plug of platelets enmeshed in a network of insoluble fibrin molecules. When blood vessels are cut or damaged, the loss of blood from the system must be stopped before shock and possible death occur. This is accomplished by solidification of the blood, a process called coagulation or clotting.[171] Blood clots are healthy and lifesaving when they

165. IgA is present in low concentrations in the serum, but it is the major immunoglobulin of secretions and has a major first-line defense role in infections. Two IgA molecules are linked by a polypeptide called the secretory piece and by a J chain. Secretory IgA is present in nonvascular fluids, such as saliva, bile, synovial fluid, and intestinal and respiratory tract secretions. Both secreted and circulating IgA types are known to have antiviral properties; their production is preferentially stimulated by local administration of antigens such as oral and aerosol immunizations.

166. IgD is found in trace quantities in the serum in humans and chickens. Its function is uncertain.

167. IgE is present in low levels in serum and is generally present in increased levels in individuals with allergy. It has not been found in the chicken. Following exposure to antigen (allergen), and its IgE binding to the Fab of two adjacent molecules, perturbations of the cell membrane are produced, leading to the release of vasoactive amines (histamine, serotonin, etc.) which are the mediators of anaphylaxis and atopic reactions, including urticaria, asthma, hayfever and gastroenteritis. Allergic reactions (urticaria, atopy, anaphylactic shock, etc.) are examples of IgE-mediated reactions. In humans and dogs there is an inherited predisposition for certain individuals to produce IgE.

168. IgG is the most abundant of the five classes of immunoglobulins. It is the only that crosses the placenta and is the major component of passive maternal antibody transfer via colostrum and yolk. It is the major antibody in the secondary humoral response of immunity.

169. IgM is the first antibody produced in the primary immune response. Like the IgG, IgM bound to antigen activates the complement system. These two immunoglobulins are specific antitoxins against the toxins of diphtheria, tetanus, snake venoms, botulism and anthrax microorganisms, and are used in defence against most infectious diseases.

170. For more information, see among others: Fahey J. L. (1965), "Antibodies and ImmunoglobulinsII. Normal Development and Changes in Disease", *The Journal of American Medical Association* (JAMA), 194 (3): 255-258; Johansson S. G. O., M. B. Uppsala (1967), "Raised levels of a new immunoglobulin Class (Ignd) in Asthma", *The Lancet*, 290 (7523): 951–953; Butler J. E. (1969), "Bovine Immunoglobulins: A Review", *Journal of Dairy Science*, 52 (12): 1895–1909; Kalpaktsoglou P. K., R. Hong, R. A. Good (1973), "The five classes of immunoglobulins in normal C3H and BALB/c mice", *Immunology*, 24 (2): 303–314; Ogra S. S., P. L. Ogra (1978), "Immunologic aspects of human colostrum and milk: I. Distribution characteristics and concentrations of immunoglobulins at different times after the onset of lactation", *The Journal of Pediatrics*, 92 (4): 546–549; Rose M. E., E. Orlans (1981), "Immunoglobulins in the egg, embryo and young chick", *Developmental & Comparative Immunology*, 5 (1): 15–20; Hombach J., T. Tsubata, L. Leclercq, H. Stappert, M. Reth (1990), "Molecular components of the B-cell antigen receptor complex of the IgM class", *Nature*, 343: 760 – 762; Boulinier T., V. Staszewski (2008), "Maternal transfer of antibodies: raising immuno-ecology issues", *Trends in Ecology & Evolution*, 23 (5): 282–288; Schroeder H. W. Jr., L. Cavacini (2010), "Structure and function of immunoglobulins", *Journal of Allergy and Clinical Immunology*, 125 (2): S41-S50.

171. Miller J. L. (1996), *Blood Coagulation and Fibrinolysis, Clinical Diagnosis and Management by Laboratory Methods*, edt. by J. B. Henry, 19th ed., W. B. Saunders Co., Philadelphia; Hardaway R. M., C. H. Williams (1996), "Disseminated Intravascular Coagulation: An Update", *Comprehensive Therapy*, 22 (11): 737-743.

stop unwanted bleeding. However harmful blood clots can also form, causing serious damage. Most heart attacks and strokes result from the sudden formation of a blood clot on a cholesterol plaque inside an artery in the heart or brain. The process of blood clotting is triggered whenever the normal flow of blood is exposed to certain substances; there are a lot of different substances, called thrombogenic substances that promote formation of thrombus (another name for a clot). These substances are located in the skin or in blood vessel walls. Examples of these thrombogenic substances are tissue factor, collagen, and von Willebrand factor. While the formation of clot is essential to life and can be regulated, inappropriate clot formation, especially in the brain or lungs, can be life-threatening.

There are many substances that inactivate the thrombin. Antithrombin III is one of these. It is a plasma protein (a serpin) that inhibits the formation of thrombin. Heparin is another inhibitor. It is a mixture of polysaccharides that bind to antithrombin III, inducing an allosteric change that greatly enhances repression of thrombin synthesis.

Fibrinolysin

It is an enzyme derived from bovine plasmin or extracted from cultures of certain bacteria. It is used in commercial preparations only and exclusively together with the enzyme desoxyribonuclease (extracted from bovine pancreas). Fibrinolysin and desoxyribonuclease both act as lytic enzymes. Fibrinolysin is thought to break down fibrin in necrotic material, while desoxyribonuclease is thought to degrade DNA residue of necrotic cells.[172] No long-term clinical benefit was demonstrated in reducing purulent exudates or necrotic tissue.[173]

Serotonin[174]

It is found mainly in the gastrointestinal tract of animals. About 80 to 90% of the human body's total serotonin is located in the enterochromaffin cells in the gut, where it is used to regulate intestinal movements. Serptonin is a monoamine neurotransmitter that regulates mainly metabolism, mood,[175] and appetite and appears to be involved in control of sleep, memory and learning, temperature regulation, behavior (including hallucinogenic behavior and sexual), cardiovascular function, muscle contraction, endocrine regulation, and depression. Almost all the body's serotonin is used by muscles and blood vessels. It is synthesized in brain neurons, from the amino acid L-tryptophan, and is stored in vesicles. Serotonin is found not only in animals, but also in fungi and plants, including fruits and vegetables.

172. Westerhof W., F. C. Jansen, F. S. De Wit, R. H. Cormane (1987), "Controlled double-blind trial of fibrinolysin –desoxyribonuclease (Elase) solution in patient with chronic leg ulcers who are treated before autoluogos skin grafting", Journal of American Acade Dermatology, 17 (1): 32-39

173. Shai A., H. I. Maibach (2005), *Wound Healing and Ulcers of the Skin: Diagnosis and Therapy - The Practical Approach*, Spring-Verlag, Berlin-Heidelberg-New York, p. 128.

174. Müller C. P., B. L. Jacobs, Edtr. (2010), *Handbook of the Behavioral Neurobiology of Serotonin*, Volume 21, Elsevier B.V., pp. 3-818.

175. Animal research suggests that central serotonergic neurons are involved in behavioral suppression, particularly anxiety-related inhibition. The hypothesis linking decreased serotonin transmission to reduced anxiety as the mechanism in the anxiolytic activity of benzodiazepines conflicts with most clinical observations (See: Soubrié P. (1986), "Reconciling the role of central serotonin neurons in human and animal behaviour", *Behavioral and Brain Sciences*, 9: 319-335).

Shi Wu Wen and others[176] have studied the safety of the use of selective serotonin reuptake inhibitors in pregnancy. They claim that the risks of low birth weight, preterm birth, fetal death, and seizures were increased in infants who were born to mothers who had received selective serotonin reuptake inhibitor therapy.

Kallikreins

They are enzymes capable of cleaving peptide bonds in proteins. They are a subgroup of serine proteases. Any of two groups of serine endopeptidases that are widely distributed in mammalian tissues and body fluids, including blood. In 1934, Werle[177] reported finding great amounts of the substances in the pancreas of human men and other animals, such that the pancreas could be taken for its site of origin. He named it kallikrein from the Greek word for pancreas. Later, Werle et al.[178] identified kallikrein as a proteolytic enzyme ('ferment') that liberates the biologically highly active basic polypeptide 'DK', or kallidin (i.e lys-bradykinin) from a blood plasma protein called kallidinogen or kininogen (H- and L-kininogen).

Human tissue kallikrein-related peptidases (KLKs) are a family of 15 serine proteases with diverse physiological functions. The vital importance of the kallikrein-kinin system for basic mechanisms in biochemistry, patho/physiology, pharmacology and more recently, molecular biology and cell biology, and the great interest and practical benefit of the system to clinical medicine, has stimulated scientists from various disciplines worldwide to become involved in kallikrein-kinin research. KLKs play important roles in different physiologic processes, such as regulation of cell growth and differentiation, tissue remodeling, skin desquamation, and human semen liquefaction,[179] etc. Several members of the human tissue kallikrein-related peptidase (KLK) family are emerging cancer biomarkers. The aim of this study was to analyse the expression of a panel of KLKs in colorectal cancer and to find out if the multiparametric combination of them can increase the accuracy of prediction of patients survival beyond the traditional clinical information.[180]

Plasmin

Like trypsin, it belongs to the family of serine proteases. Plasmin is an enzyme present in blood that degrades many blood plasma proteins, particularly, fibrin clots. The generation of plasmin by plasminogen activators is a physiologic process in animals that dissolves blood clots and promotes wound healing, blood vessel growth, and the migration of normal and

176. Wen S. W., Q. Yang, P. Garner, W. Fraser, O. Olatunbosun, C. Nimrod, M. Walker (2006), "Selective serotonin reuptake inhibitors and adverse pregnancy outcomes", *American Journal of Obstetrics and Gynecology*, 194 (4): 961–966.
177. Werle E. (1934), "Zur Kenntnis des Haushalts des Kallikreins", *Biochemische Zeitschrift*, 269: 415-434.
178. Werle E., W. Götze, A. Kappler (1937), "Über die Wirkung des Kallikreins auf den isolierten Darm und über eine neue darmkontrahierende Substanz", *Biochemische Zeitschrift*, 289: 217-233.
179. Pampalakis G., G. Sotiropoulou (2007), "Tissue kallikrein proteolytic cascade pathways in normal physiology and cancer", *Biochimica et Biophysica Acta*, 1776 (1): 22–31.
180. Talieri M., L. Li, Y. Zheng, D. K. Alexopoulou, A. Soosaipillai, A. Scorilas, D. Xynopoulos, E. P. Diamandis (2009), "The use of kallikrein-related peptidases as adjuvant prognostic markers in colorectal cancer", *British Journal of Cancer*, 100 (10): 1659–1665.

cancerous cells.[181] Streptokinase and urokinase have been the standard agents available for many years, but in recent years the most exciting change in the field has been the development of a new generation of plasminogen activators, the principal one being a tissue plasminogen activator.[182] A tissue plasminogen activator is a clot-dissolving enzyme that is naturally produced by cells in the walls of blood vessels; it catalyzes the conversion of plasminogen to plasmin. Plasmin is the main proteolytic enzyme in milk and has been found to be associated with enhanced casein hydrolysation.[183]

The role of the plasminogen-plasmin system in fertilization is unknown, although its dysfunction has been associated with subfertility in humans. Coy and coll.[184] have recently detected and quantified plasminogen in the oviductal fluid of two mammals and showed a reduction in sperm penetration during IVF when plasminogen is present. The objective of this study was to describe the mechanism by which the plasminogen-plasmin system regulates sperm entry into the oocyte.

According to Marder and co-workers,[185] plasmin is tolerated without bleeding at a several-fold higher amount than that needed for thrombolysis, which is in contradistinction to plasminogen activators that risk bleeding at any effective thrombolytic dose. Plasmin has been safe in a current trial in patients with peripheral arterial or graft occlusion, and efforts are now directed toward use in therapy of stroke caused by cerebral artery occlusion.[186]

Saa and coll.[187] have undertaken a study to examine the effect of plasmin on sperm viability and sperm–oocyte interaction during in vitro fertilization in the pig. The results suggest that plasmin might play a role in events related to fertilization.

g) Veterinary biological uses (also called veterinary biologics) are those used for the diagnosis and treatment of diseases in animals including vaccines, bacterins, antitoxins, toxoids, immunomodulators.

181. Gladysheva I. P., R. B. Turner, I. Y. Sazonova, L. Liu, G. L. Reed (2003), "Coevolutionary patterns in plasminogen activation", *PNAS*, 100 (16): 9168-9172.

182. Hirsh J., A. G. Turpie (1990), "Use of plasminogen activators in venous thrombosis", *World Journal of Surgery*, 14 (5): 688-93.

183. Battacone G., E. A. Cannas, A. Mazzette, C. Dimauro, G. Enne (2005), "Why does the increase of plasmin worsen the coagulation properties of milk in dairy sheep?", *Italian Journal of Animal Science*, 4 (Suppl. 2): 342-344.

184. Coy P., M. Jiménez-Movilla, F. A. García-Vázquez, I. Mondéjar, L. Grullón, R. Romar (2012), "Oocytes use the plasminogen-plasmin system to remove supernumerary spermatozoa", *Oxford Journals - Human Reproduction*, 27 (7): 1985-1993.

185. Marder V. J., R. Jahan, T. Gruber, A. Goyal, V. Arora (2010), "Approaches to Thrombolysis. Thrombolysis With Plasmin. Implications for Stroke Treatment", *Stroke*, 41: S45-S49.

186. See also: Riviere J. E., M. G. Papich (2009), *Veterinary Pharmacology & Therapeutics*, Wiley-Blackwell, 9$_{th}$ Edition, pp. 685-6.

187. Saa S. J., H. H. Rheeb, H. T. Cheonga, B. K. Yanga, C. K. Park (2006), "Effects of plasmin on sperm–oocyte interactions during in vitro fertilization in the pig", *Animal Reproduction Science*, 95 (3–4): 273–282.

h) Industrial uses

Adhesives

They may be defined as any substance capable of attaching materials together by means of surface attachment. Knowledge and use of adhesives is very old. The ancient Egyptians, 3500 years before Christ, knew well the art of veneering and used adhesives to attach decorations to wood. Clay, mud, and dung, along with mixtures of these substances, must also be regarded as adhesives and have been used for centuries. Until the Second World War, essentially all of the glues were of natural origin. Adhesives based on synthetic polymers were introduced just before that war and now surpass most of the older natural glues in importance for wood bonding.[188]

Fish skin, bones, horns and hides of cows, seal brain and blood are a little part of the list of animals used for manufacturing glues. As a consequence, animal glue includes hide glue, bone glue, fish glue, blood glue and skin glue. They are generally classified into three main types: those made from hide and bone, those made from fish skins, and those made from fresh or dried beef blood.[189] Hide glue is used in woodworking. Besides horses, hide glue uses cows' hooves, bones, and hides.

Blood glues are either made from fresh animal blood or soluble dried beef blood, a by-product of the meat packaging operation. The latter is produced by evaporating the serum from fresh whole blood. The adhesive can be made from any animal blood, such as bovine, porcine, or avian blood.

Water-resistant glues based on blood albumen are made by mixing the dried blood powder, obtained from cattle or hog slaughter houses, with water which is then activated by the addition of an alkali such as slaked lime and caustic soda and perhaps other chemicals such as ashes, and/or alum.

Another type of adhesive is made by adding an anticoagulant and a preservative to fresh, whole animal blood without dewatering the blood. Lime is then added to the anti-coagulated, preserved blood, and the pH is adjusted to 9-11. A curing agent (such as potassium silicate, sodium silicate and aluminum dihydrogen phosphate) and ammonia are then added to the mixture to yield the animal blood adhesive.[190]

Blood glues have been used as plywood adhesive, as paint for brickwork, as adhesive for filling joints between brick and building stones, and in food packaging since the bonds are odorless, nontoxic, and tasteless.[191] A typical use was the bonding of cork disks in metal bottle caps. In some countries, blood glues made of cow's blood are used for plywood manufacture.

Animal blood can also be used to make water-soluble ketone-aldheyde and phenol-

188. The introduction of synthetic resin glues proved more convenient to use and some of them excelled the blood albumin glues in durability under severe service conditions.

189. Blood glues may be made either from the fresh blood of slaughtered animals or from the dried soluble blood albumin. To make the use of fresh blood feasible, the supply must be readily accessible to the place of manufacture, inasmuch as rapid decomposition takes place and renders it unsatisfactory for its purpose. Alternatively you can use the fresh blood treated with a preservative.

190. Gunasekaran S., H. Lin, *Glue from slaughterhouse animal blood*, United States Patent Application 20100018436.

191. See: Hubbard J. (1977), *Animal Glues in Handbook of Adhesives*, I. Skeist (ed.), Van Nostrand Reinhold, New York, pp. 172-180.

ketone-aldheyde resin binders become more extendable and more sprayable.[192]

These glues have quite good water resistant qualities but under damp conditions are highly susceptible to mold growth and attack by bacteria.

The use of dural sealants has become common in neurosurgery. One of these is represented by a bovine albumin-glutaraldehyde combination (BioGlue[193]). Klimo and al., however, have discovered that ten patients implanted with that adhesive have had wound complications.[194]

A two-component sealant composed of bovine serum albumin and glutaraldehyde (BioGlue) is used to treat aortic dissections. However, some studies[195] show that polymerized BioGlue releases amounts of glutaraldehyde that are capable of inducing cytotoxic effects both in vitro and in vivo. Use of BioGlue should be restricted to the aortic dissection procedure, as other tissues are sensitive to the amounts of glutaraldehyde released from the glue.

Research[196] conducted in the USA confirm that autologous fibrinogen, derived by polyethylene glycol precipitation from the blood of an individual patient would avoid the risk of transmitting hepatitis, and it has been shown to be relatively safe in animal studies. Fibrinogen-based adhesive, derived from pooled human plasma, has been used in Europe with great success in otologic surgery, but has not been approved for use in the U.S.

The above reports and others suggest that fibrin glue can be used to seal porous vascular grafts prior to insertion. Gundry and Behrendt[197] have studied that ability comparing blood loss from and handling characteristics of grafts pretreated either with fibrin glue, albumin autoclaving, or blood preclotting. They concluded that "fibrin glue or albumin is superior to blood for pretreatment of woven grafts in limiting blood loss. Fibrin glue imparts superior handling characteristics".

Experiments with medical glues suggest that one-third of all wounds may be "stitched" with glues in the next few years. The adhesive have the potential to be used in tens of thousands of operations in which the delicacy of the surgery rules out the use of sutures and staples.

For many applications natural adhesives have been replaced by synthetics; however, animal glues, starches, gums, natural rubber cements, bitumens, and cellulose continue to be used in large volumes. Modern synthetic adhesives do not require animal lives, and offer

192. See for example: *Blood albumin glues: their manufacture, preparation and application*, US Department of Agriculture. Forest Service. Report n. 281-2, 1955, Wisconsin – USA; Campbell C. C., Cherry Hill (1969), *Acetone-Formaldehyde and Phenol-Acetone-Formaldheyde extended with animal blood or soy flour and oil-in-water emulsion by*, US Patent n. 3,471,420 of October 7, 1969.

193. BioGlue is used to help seal leaks around sutures (surgical stitches) or staples in large blood vessels such as the aorta or the femoral and carotid arteries. Product name is CryoLife BioGlue Surgical Adhesive.

194. Klimo P. Jr., A. Khalil, J. R. Slotkin, E. R. Smith, M. R. Scott, L. Goumnerova (2007), "Wound Complications Associated With the Use of Bovine Serum Albumin-Glutaraldehyde Surgical Adhesive in Pediatric Patients", *Neurosurgery*, 60 (4): 305-309.

195. Fürst W., A. Banerjee (2005), "Release of Glutaraldehyde From an Albumin-Glutaraldehyde Tissue Adhesive Causes Significant In Vitro and In Vivo Toxicity", *The Annals of Thoracic Surgery*, 79: 1522-1528.

196. Weisman R. A., A. J. Torsiglieri, A. D. Schreiber, G. H. Epstein (1987), "Biochemical characterization of autologous fibrinogen adhesive", *The Laryngoscope*, 97 (10): 1186–1190.

197. Gundry S. R., D. M. Behrendt (1987), "A Quantitative and Qualitative Comparison of Fibrin Glue, Albumin, and Blood as Agents to Pretreat Porous Vascular Grafts", *Journal of Surgical Research*, 43 (1): 75-77.

several other advantages: longer shelf lives, lower freight costs, reliability and flexibility of application. In addition, synthetic adhesives find applications in the textiles, paper, dyeing, printing, furniture, plumbing, shoe, book, building, and automobile sectors. Many communities should be using them in order not to violate their religious and moral principles. For example, in India animal glue is still used in the manufacture of matches, books, textiles, cycles, and sports goods. The traditional Indian forehead "Bindi", the emery nail file, sports racquet grips, and even shoes termed as "non-leather" are sometimes glued using parts of a cow.

The United States is a major producer and consumer of glued-wood products and therefore of adhesive resin solids.[198] To manufacture about 60 million cubic meters/year, the adhesive resin solids required to bond glued-wood products are estimated to be about 1.5 million metric t/year. Nearly 60% of adhesive consumptions are UF (urea-formaldehyde), about 30% are PF (phenol-formaldehyde) and RF (resorcinol-formaldehyde), and the remaining 10% consist of several products, including PMDI (diphenyl methylene diisocyanate).

Increasing oil prices are boosting resin prices. Adhesive are a vital part of glued-wood composites, their cost range contributing about 30% of the finished product. As a consequence research continues to seek natural alternatives that can effectively replace synthetic resin adhesives as a wood binder.

Casein is perhaps the first structural adhesives, and it is still in use. Production volume is small (under 5000 t/year). Most is modified with soy flour. Soybean glues and soy-modified casein adhesives have a long history, and, because of the green movement in costruction, there is renewed interest in soy flour and soy protein isolates for wood-based and agri-fiber composites. Research continues on soy products for finger jointing adhesives, as replacements for animal blood in plywood production, for PF and PMDI resin blends in OSB, and as a wheat/soy blends for plywood glue extenders. In North America, soluble animal blood is now rare as an adhesive, but it is indispensable in PF foam adhesives for industrial and construction plywoods. Eleven mills in North America and one in Europe were using foam glues in 1999.

Finishes for leather and textiles

Ox blood is used as a non-thermoplastic binder in finish preparations. Added to black or dark coloured leather, it improves the leather's depth of colour and brilliance.

The blood's fibrin fibers appear to stretch farther than any other natural fiber, up to six times their length, before breaking according to an expert's report in 2006.[199]

According to Miyata and Taira[200] collagen is a typical biological macromolecule having been often utilized as a material with properties similar to cellulose. Nowadays its application is becoming more widespread, ranging from classical applications such as in the leather, gelatin, and food industries to more current uses in the biomaterial and biotechnological fields.

198. Orr L. (2007), *Wood Adhesives. A market opportunity study*, USB, Midland (Mi - USA).

199. Liu W., L. M. Jawerth, E. A. Sparks, M. R. Falvo, R. R. Hantgan, R. Superfine, S. T. Lord, M. Guthold (2006), "Fibrin Fibers Have Extraordinary Extensibility and Elasticity", *Science*, 313 (5787): 684-687.

200. Miyata T., T. Taira (1992), "Collagen engineering for biomaterial use", *Clinical Materials*, 9 (3–4): 139-148.

Foam fire extinguisher [201]

In about 200 BC, the Roman Ctesibius of Alexandria is credited with inventing a hand operated fire pump able to deliver a stream of water to a fire. It was the precursor for other variants of forcing water out of a container.

Subsequently the water inside the fire extinguisher was replaced with foam capable of controlling flammable solid fires (substances such as wood, paper, hay, etc.), and fires fueled by flammable oils, petrol, diesel, spirits etc. A foam fire extinguishers now contains chemical foam, but in the past it used to contain ox blood. Ox blood inside the extinguisher reacted with CO_2 to create a foam that, under pressure, was released to form a sealing cover over a liquid fire. The heat from the fire would "cook" the foam forming a blanket on the surface of the burning liquid or solid; this ox blood blanket cooled and smothered the fire in such a way that no oxygen or heat was available to re–ignite the fire.

When protein compounds are prepared by the chemically treating various protein materials are then suitably diluted with water, they produce stable foam for fire fighting purposes. The basic materials used in the manufacture of protein fire-fighting compounds are horn and hoof meal, and animal blood. During the course of manufacture of horn and hoof meal a certain amount of acid is used. In earlier times the acid was sulphuric. Compounds made by this process tended to the formation of sludge, consisting mainly of crystals of calcium and sodium sulphate. This compound, referred as a "sulphate compound", is no longer manufactured. To overcome the sludging problem, hydrochloric acid was used and this compound is known as "chloride compound".

A foam fire extinguisher must be non toxic, non damaging to most materials, must extinguish fires progressively, and must prevent the re – ignition of flammable liquid fires.

Since 2003, University of Alberta (USA) scientists have been working with the agriculture industry to transform protein from the blood of slaughtered cows into a new fire fighting foam. The impetus for the research was the 2003 Mad Cow crisis, which adversely impacted many cattle-by product markets. Prior to that crisis, blood meal had been sold as an additive to livestock feed. After the crisis, chemical companies identified a potential market application for a protein-based foaming agent. Blood meal is high in protein that stabilizes foam. Foam extinguishes fires. The research grew from there.

Porous concrete [202]

Concrete is a strong, hard, building material composed of sand, gravel, cement, and water. Concrete additives have been used since Roman and Egyptian times, when it was discovered that adding volcanic ash to the mix allowed it to set-up under water. Similarly, the Romans knew that adding horse hair made concrete less liable to crack while it hardened

201. Ratzer A. F. (1956), "History and Development of Foam as a Fire Extinguishing Medium", *Industrial & Engineering Chemistry*, 48 (11): 2013–2016; Rivkind L. E., I. Myerson (1956), "Foams for Industrial Fire Protection", *Industrial & Engineering Chemistry*, vol. 48 (11): 2017–2020; Tuve R. L., H. B. Peterson (1956), "Characterization of Foams for Fire Extinguishment", *Industrial & Engineering Chemistry*,. 48 (11): 2024–2030; Perri J. M., C. Conway (1956), "Foam as a Fire Exposure Protection Medium—Evaluating Effectiveness of Wetting and Protein Agents", *Industrial & Engineering Chemistry*, 48 (11): 2021–2023.

202. Sedgwick J. (1988), "Strong but sensitive", *Atlantic Monthly 1991-4*, 267 (4): 70-82; Weisburd S. (1988), "Hard Science", *Science news*, 134 (2): 24-26.

and adding blood to strengthened it against frost. Concrete is a porous material and adding larger pored blood to the mix allows moisture to expand into the blood cells deterring concrete cracks when temperature drop. Today, though the same ancient principles apply, polymer fibers are used instead of horse hair and specialist "air-entraining agents" are used instead of blood.

Plastic and cosmetic base formulations

The use of cosmetics, since ancient times, has been widespread in the societies that want to improve appearance (e.g., by highlighting certain features of the face and/or accentuating natural colors) or to hide imperfections of the skin. Cosmetics usually refer to substances or preparations intended for contact with the epidermis of the human body in order to provide functions such as cleansing, protecting, changing appearance, perfuming, altering the odor, and improving or maintaining skin conditions. More specific functions for cosmetics are the following: cellulite reduction, edema reduction, sebum removal, exfoliation/peeling, anti-aging, anti-wrinkle-pucker, anti-acne-zits-pimples/spots, moisturizing or lubricating of skin, anti-clogging of pores, coloring/tanning.[203]

Many raw materials with high protein content (e.g. proteins from wheat, soybean, sunflower seed, peanut, rapeseed, etc., fish or meat proteins, seric albumin or egg, casein, blood, collagen, etc.) can be used to manufacture different products, with different functional properties (adhesive, thermoplastic, elastomeric, thermoset, cosmetic, etc.). There is considerable potential for modulating the properties of protein based materials because of the variability of their amino-acid composition. Products made from proteins are usually biodegradable and sometimes even edible when food-grade components and manufacturing processes are used.[204] Cosmetics can contain albumin, a protein component of blood. Animal byproducts, such as blood can also be used to formulate cosmetics.

In 1964, a particular cosmetic ("Magic Secret") was introduced into the US market. The product was advertised as a wrinkle-smoothing lotion that "smooths away wrinkles in minutes, keeps them away for hours". Magic Secret was followed by many similar cosmetic creams, with the same features. All of these products were based on the bovine serum albumin extracted from cattle blood. One of the advantages of using blood serum as an albumin source was that it was very easy to extract.

The albumin is water soluble and light in color; its concentration (albumin content) is about 80-95%. Bovine albumin is available from several sources in three forms: as a 15% sterile solution ready for immediate use without dilution; as a 30% solution to be diluted with an equal volume of water prior to use, and also in the form of a freeze-dried powder which is reconstituted with water before use.

203. De Navarre M. G. (1975), *The chemistry and manufacture of cosmetics*, (2nd. ed., Vols. I-IV), Continental Press Inc., Orlando (PA - USA).
204. Guilbert S., Marie-H. Morel, N. Gontard, B. Cuq (2006), *Protein-Based Plastics and Composites as Smart Green Materials*, ACS Symposium Series, vol. 921, Chapter 24, pp 334–350.

4.3 Bones

Bone material is a mixture of calcium phosphate and calcium carbonate with other minerals present. The calcium lies in the form of a mixture of hydroxyapatite (HAP) crystals[205] and amorphous calcium phosphate. The largest fraction of hard bone is found in the high-density leg bones of cattle, sheep, and goats. Poultry bones are essentially all soft bones.

A natural form of HAP has applications as an absorbent, a catalyst, a dental substrate, and as a bone substitute. Specially processed cartilage from the breast-bone of young cattle is used by plastic surgeons to replace facial bones in human beings. In most instances, the hard animal bones are ground into bone-protein meal and used as a source of calcium.

HAP is a crystalline material that is water insoluble. Its chemical properties and physical structure make HAP useful as chromatographic packing material, catalyst,[206] catalyst support, enzyme immobilization substrate, and as a component for artificial bone reconstruction.

Catalyst

The calcium in HAP can be replaced by other metals such as strontium, copper, lead, platinum, iron, barium, etc. The Ca/P ratio and the ability to substitute other metals for part of the calcium are the properties of HAP that influence its catalytic performance. In the scientific literature there are a lot of reports about catalysts based on HAP with partial substitution of the calcium by platinum, copper, cobalt, nickel, etc. The applications of HAP as catalysts include HAP with Pt, Co, Cu, Fe, Ni useful as exhaust gas catalyst to remove NOx, CO_2 and Hydrocarbons; HAP and acidic HAP useful for partial oxidation of methane to CO and H_2 (to use for fuel cells) or to produce formaldehyde from methanol; for amination of alcohols; for dehydrogenation of propane to propylene; for production of ethylene from methane (HAP with Pb); and for conversion of aromatics to alkyl aromatics (HAP with Zn, Ni, $CuCl_2$).

The market for solid catalyst car exhausts and fuel cells is a high value-added area and seems set for future growth on the back of exponential growth in the nanotechnology sector. There is prior art in the use of synthetic HAP as a catalyst support.

Application for biological implant[207]

Ceramics used for restoration of teeth and bones are often formed by a mixture of whitlockite[208] and HAP. One method of using HAP is to form a polymer-mineral composite by precipitating the HAP onto polymer fibers. The use of polymer fibers allows formation of the shape needed for the prosthesis. It follows the insertion of HAP onto the polymer fibers and insert the prosthesis. In the course of time the polymer is absorbed and the HAP forms a matrix for the natural bone to grow.

205. HAP is a crystalline mineral that has the nominal formula $Ca_{10}(PO_4)_6(OH)_3$, with a stoichiometric (Ca/P) ratio of 1,67. It is prepared reacting $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$. The solids obtained are ground to the desired size for the catalyst application.

206. Catalysts are at the heart of the petrochemical industry. Their sales in refining are about 2,5 billion dollars/year, with a 2% growth rate.

207. An implant is a medical device manufactured to replace a missing or support a damaged biological structure, or enhance an existing biological structure.

208. *Whitlockite* is a form of crystalline calcium phosphate with formula $Ca_9(MgFe)(PO_4)6PO_3OH$.

It is very difficult to develop the medical market for reconstructive bone and dental applications. Perhaps non-human markets for HAP ceramics and catalysts could advance health impact concerning human contact.

If HAP could fullfill even a small application in the ceramic or catalyst market, the demand for its supply would have a considerable impact on the co-products industry.

Column packing and support application for hydroxyapatite.

As HAP crystal structure has stable hydroxyl groups, it is an ideal packing material for chromatographic applications. Applications include ion exchange substrate protein enrichment-recovery by column chromatography.

Glues

They are made by boiling down the hooves of hooved animals such as horses, deer and donkeys. The process is to break the hooves into small chunks and then boil the chunks in water until all the hoof material has been liquefied. An acid is then added to create a thick gel. The resultant product is then cooled and allowed to harden. Hoof glues have been used for things like stiffening bow strings, stiffening and adhering fabric to wood, creating thin lacquers to protect valuable objects as well as sealing glass into frames and sealing ceramic containers. It is still used today in woodworking, specifically cabinetry.

Bone China

Cattle bones, transformed into fine "Bone China", have forayed into the finest dinner services and tea sets imaginable. Bone China is a type of porcelain that is composed of bone ash, kaolin and feldspathic material.[209] Thomas Frye first developed Bone China at his factory near Bow (London) in 1748. His factory was located very close to the cattle markets and slaughterhouses of Essex, and hence he had easy access to animal bones. Frye used up to 45% bone ash in his formulation. Others have said that Bone China was invented by the by the Chinese in the seventh and eighth centuries B. C. and improved by Frye. Still others[210] believe that the development of Bone China should be credited to Josiah Spode, who introduced it in 1797. Soon afterwards Bone China formulas were copied by factories in Liverpool, England.

Nowadays Bone China contains 35-45% bone ash in the clay formula, making the major component a renewable resource.

4.4 Brains and spinal cords

For table use

The brain is usually prepared direct for the table rather than processed for industrial use. Because of its soft texture, brain is blanched to firm the tissue before cooking. The membranes (the arachnoid meninges and pia mater) are peeled from the brain before cooking. In addition, brains are a source of cholesterol that is the raw material for the synthesis of

209. "Production of Bone Ash for the Manufacture of Bone China", *Industrial Ceramics* 1989, 843, p . 767-770.

210. Karwatka D. (2009), "Josiah Spode and His World-Famous Pottery", *techdirections*, 68 (9): 12; Badenhorst A. (2010), "Porcelain", *Ceramics Technical*, 3: 34.

vitamin D_3 and can be used as emulsifier in cosmetics. Other materials can be isolated from the hypothalamus of the brain.

Clathrin

Cow brains could possibly be useful in creating batteries, electrodes and solar cells. Clathrin is present in every cell in the human body, and cows possess a vast wealth of it in their bovine brains, making them an ideal source for the stuff.[211] Clathrin molecules are capable of forming tiny cubes, spheres, and other shapes in test tubes.[212] Inorganic materials can like gold and titanium dioxide can be added to the clathrin molecules.[213] Some of these materials have been found to be able to absorb sunlight, or to split water into its components.

Melatonin

The hormone melatonin, extracted from the pineal gland, is being evaluated for the treatment of schizophrenia, insomnia, and other problems[214] including mental retardation.

Fukui, Takagi and others[215] have isolated a novel analgesic peptide from the bovine brain.

211. To know more about see: Lindner R., E. Ungewick (1992), "Clathrin-associated Proteins of Bovine Brain Coated Vesicles. An analysis of their number and assembly-promoting activity", *The Journal of Biological Chemistry*, 267 (23): 16567-16573; Ahle S., E..Ungewickell (1990), "Auxilin, a newly identified clathrin-associated protein in coated vesicles from bovine brain", *The Journal of Cell Biology*, 111 (1): 19-29.

212. Heilshorn S., N. Melosh, S. Doniach, A. Spakowitz (2011), *Protein Biotemplates for Self-Assembly of Nanostructures*, in Proceedings of the *2011 Biomolecular Materials Principal Investigators' Meeting* held on October 23–26, 2011 at the Westin Annapolis Hotel in Annapolis, (MD-USA), pp. 26-29; VanDersarl J., S. Mehraeen, A. Schoen, S. Heilshorn, A. Spakowitz, N. Melosh (2011), *Mechanics of 2-D Clathrin Assembly*, Proceedings of the *2011 Biomolecular Materials Principal Investigators' Meeting* held on October 23–26, 2011 at the Westin Annapolis Hotel in Annapolis, (MD-USA), p. 243.

213. Arora H., C. Doty, Ye Yuan, J. Boyle, K. Petras, B. Rabatic, T. Paunesku, G. Woloschak (2011), *Titanium Dioxide Nanocomposites*, in *Nanomaterials for the Life Sciences*, Vol.8: *Nanocomposites*, Edited by Challa S. S. R. Kumar, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; Heilshorn S. C. (2010), *Self-Assembling Materials for Energy Storage and Transport,* 55th Annual Report on Research 2010, Reports: DNI10.

214. People use melatonin to adjust the body's internal clock, i. e. for jet lag, for adjusting sleep-wake cycles (shift-work disorder), and for helping blind people establish a day and night cycle. It is also used for the insomnia; delayed sleep phase syndrome; insomnia associated with attention deficit-hyperactivity disorder; insomnia due to certain high blood pressure medications called beta-blockers; and sleep problems in children with developmental disorders including autism, cerebral palsy, and mental retardation. Some people use melatonin for Alzheimer's disease, ringing in the ears, depression, chronic fatigue syndrome, fibromyalgia, migraine and other headaches, irritable bowel syndrome, osteoporosis, a movement disorder called tardive dyskinesia, epilepsy, as an anti-aging agent, for menopause, and for birth control. Other uses include breast cancer, brain cancer, lung cancer, prostate cancer, head cancer, neck cancer, and gastrointestinal cancer.

215. Fukui K., H. Shiomi, H. Takagi, K. Hayashi, Y. Kiso, K. Kitagawa (1983), "Isolation from bovine brain of a novel analgesic peptapeptide, neo-kyotorphin, containing the Tyr-Arg (kyotorphin) unit", *Neuropharmacology*, 22 (2): 191–6; Takagi H., H. Shiomi, K. Fukui, K. Hayashi, Y. Kiso, K. Kitagawa (1982), "Isolation of a novel analgesic pentapeptide, neo-kyotorphin, from bovine brain", *Life Sciences*, 31 (16–17): 1733–1736.

Spinal cord: factor B

As we know, the spinal cord is a long, tubular bundle of nerve tissue and support cells that extends from the brain lengthwise along the back within the vertebral canal. Pairs of spinal nerves, originating in the spinal cord, carry impulses to and from the brain. Spinal cord-derived growth factor-B is a member of the platelet-derived growth factor family.

Cholesterol

Cholesterol is a significant lipid in animal fats and the most abundant steroid. It can be extracted from the spinal cord of animals and used in production of vitamin D and sex hormones. These compounds have multiple functions in biochemical systems acting as lipids (e.g. cholesterol), surfactants (the bile acids), and hormones etc.

4.5 Fats and fatty acids

Animal fats are made mainly from triglycerides. The product from rendering constitute about 25% of the lipid resources used in the world. The main use of animal fats and oils are for human and animal nutrition; the fats and oil are a dietary source of energy.

The unsaturated fatty acids (linoleic, linolenic, oleic and arachidonic acid) are nutritionally important as they are necessary constituents of cell walls, mitochondria and other intensively active metabolic sites of the living organism.

In recent years it has been suggested that a high ratio of unsaturated to saturated fatty acids in the diet is desirable as this may lower the individual's susceptibility to cardiovascular diseases. There is indicating evidence that a diet relatively high in saturated fats (such as those of found in red meat) raises the level of cholesterol in the blood.

The fat content of animal carcasses vary from about 8 to 20% (the latter only in pigs). The fatty acid composition of the fatty tissues is very different in various locations of the body. For example, external fat ("body fat") contains a higher concentration of unsaturated fat and is much softer than the internal fat surrounding organs, which has a high saturated fat content. In pigs, the subcutaneous[216] fats are separated from other tissues and are suitable for feeding, industrial uses and for meat processing (backfat, jowl fat and belly). The kidney fat and leaf fat of pigs are used for lard production, as they are not recommended as an ingredient for processed meat products because of their hardness and taint.

Beef fats (tallow) are considered less suitable for further processing than pig fats due to their firm texture and intense flavour. If used for processing, preference is given to brisket fat and fats from young animals. Such fats are used for specific processed beef products.

Some tropical cattle breeds have a large subcutaneous fat depot, known as "hump", in the shoulder region, that is often cut into slices and roasted as a delicacy, and sometimes used for processed products. Buffalo fat has a white colour and is well suited for processing. The limiting factor for utilization of tropical beef and buffalo fat is scarce availability.

Almost always mutton fat of adult animals is absolutely unsuitable for consumption due to its intensive unpleasant flavour and taste. Fats from lamb are relatively neutral in taste and commonly eaten with meat.

Chicken fat has a neutral taste and is suitable as a fat component for pure chicken prod-

216. Subcutaneous and intermuscular fats are known as "body fats", while another category are the "depot-fats", located in the animal body around internal organs.

ucts. Chicken fat adheres to chicken muscle as intermuscular fat. The majority of chicken fat derives from chicken skin with its high subcutaneous fat content. At present these fats are underutilized render materials. They do not appear in the end use market.

The use of the animal fats in industrial applications, in Europe and in USA, on average, regards the following areas with the utilization percentage:

- plastics and plasticizers	28%
- adhesives	20%
- surfactants	15%
- solvents	15%
- industrial chemicals	12%
- lubricants	8%
- agrochemicals	2%

Industrial uses for fats and fatty acids are the following:

Antifreeze, biodegradable detergents, biodiesel, bone char to filter and decolorize sugar solutions, bone china, ceramics, chemicals, cosmetics, crayons, creams and lotions, dish and hand soap, explosives, floor wax, fuel, hair conditioner, herbicides, industrial oil and lubricants, insecticides, makeup, medicines, mink oil, oleo margarine & shortening, paints, paraffin, rubber products, saddle soap, shaving cream, solvents, tallow for tanning.

For production of energy

Historically, tallow has had much wider energy and industrial application than protein-based meals. The rendering process is not a new industry; the production of tallow for candles and soap has occurred for centuries. Soap making has made major use of tallow. In the nineteenth century the Industrial Revolution transformed the agriculture sector. The development of intensive livestock production led to a burgeoning disposal problem. Rendering became an attractive solution. Early twentieth century processes, called tankage, separated the fat and water from the protein, which was then used as a fertilizer. It was only during the the first years of the 1900s that the conversion of ABPs to meal and bone meal for animal feed became important.

The onset of World War I and II saw significant demand for rendered glycerin for the production of explosives, specifically tri-nitroglycerin (TNT). The production of meal and bone meal and tallow continued to increase after the war. According to the UK Department for Environment, Food and Rural Affairs,[217] the production and use of meal and bone meal steadily increased throughout the first half of the century. Tallow, however, was the primary product of rendering.

Tallow can be used directly as a boiler fuel or to manufacture biodiesel. There are physical and chemical methods for transforming this product into a commercial fuel. Some systems may require filtration for fats and greases before use as boiler fuel. The tallow produced by mortality rendering can be used as an alternative burner fuel. With good results, a mixture of chicken fat and beef tallow has been blended with No. 2 fuel oil in a ratio of 33% chicken fat/beef tallow and 77% No. 2 fuel oil. But the best way to get energy from tallow or fats, normally, is to turn them into biodiesel.

217. UKDEFRA (2000), *The BSE inquiry report*, Vol. 13: *Industry processes and controls*, Ch. 6 rendering, Annex B manufacturing processes of rendering (See: http://www.bseinquiry.gov.uk/report/volume13/chapterj.htm).

Despite precise written sources, the concept of using vegetal oil as an engine fuel likely dates back to Rudolf Diesel (1858-1913) who developed the first engine to run on peanut oil, which was demonstrated at the World Exhibition in Paris in 1900. Diesel believed[218] biomass fuel to be a viable alternative to the resource consuming steam engine. Vegetable oils were used in diesel engines until the 1920s when an alteration was made to the engine in order to use a fraction of petroleum. That petroleum is now known as diesel fuel (in USA diesel No. 2). In recent years, instead of diesel fuel, the Biodiesel produced from renewable sources such as vegetable oils, animal fats such as tallow,[219] and recycled cooking oils can be used in the engines.[220]

Biodiesel, defined as the mono-alkyl esters of vegetable oils or animal fats, is produced by transesterification with an alcohol in the presence of a catalyst. It is biodegradable and non-toxic, and when burned has significantly fewer emissions than petroleum-based diesel. Biodiesel functions in current diesel engines, and is a possible candidate to replace fossil fuels as a significant supplier to the world's transport energy.

Glycerol is a major by-product of the production of that reaction. The greatest attention at this time is the production of biodiesel fuel in the form of methyl esters. The process is typically catalyzed by sodium hydroxide (NaOH) or potassium hydroxide (KOH) to increase reaction rates. Biodiesel has a gross calorific value of about 33.3 MJ/l and a density of 0.88 kg/l.

A higher free fatty acid (FFA) composition of fat is likely to require more pretreatment before biodiesel production and will generate a lower quality glycerin byproduct. Higher levels of free fatty acids (FFA) generally mean lower quality and value of tallow. Commercial operations do exist that convert FFA to biodiesel in the presence of acid-based catalysts where the FFA content is less than 20 percent.

.Biodiesel is already in wide use around the world because it offers many advantages: it is in liquid form and is therefore easily stored and transported; it can be blended with diesel fuel in the same way that ethanol is blended with petrol.[221]

However, biodiesel produced from tallow presents one disadvantage: its cold flow properties. Crystallization in tallow esters (biodiesel) occurs due to the high melting points of the saturated fatty acid esters present in the biodiesel.[222] Neat (100%) methyl tallowate biodiesels have been shown to crystallize at significantly higher temperatures (melting point of methyl stearate is 39.1°C) than regular diesel (i.e., up to 15ºC).

218. "The use of vegetable oils for engine fuels may seem insignificant today. But such oils may become in the course of time as important as the petroleum and coal tar products of the present time", Rudolf Diesel, 1912 (See: Nitske W.R., C. M. Wilson (1965), *Rudolf Diesel Pioneer of the Age of Power*, University of Oklahoma Press, Norman, (OK – USA).

219. The fats produced by the rendering process can be divided into two groups: edible and inedible. Edible fats are likely to attract a higher price in the food market. Inedible rendering products attract a lower price and may be more suitable for biodiesel production.

220. Felizardoa P., M. J. Neiva Correiaa, I. Raposob, J. F. Mendesc, R. Berkemeierd, J. Moura Bordado (2006), "Production of biodiesel from waste frying oils", *Waste Management*, 26 (5): 487–494.

221. The standard blend is 20 percent biodiesel, 80 percent diesel fuel (Paisley M. A. (2001), Biomass Energy, in *Kirk-Othmer Encyclopaedia of Chemical Technology*, by John Wiley & Sons, Inc., Weinheim).

222. Papadopoulos E., S. Clarke (2005), *Modification of Tallow for Better Performance as Biodiesel*, Flinders University, Adelaide, Australia.

Several options exist for the improvement of cold flow characteristics,[223] including blending with regular diesel, use of branched chain alcohols,[224] and the use of additives.[225]

With regard to the economic aspects, the main benefit would be the conversion of low value, inedible rendered products to a higher value medium energy content fuel. The estimated operating costs of biodiesel production[226] indicate that most of the operating cost associated with typical biodiesel production is the cost of the raw material (oil/fat). The cost of methanol, labor, catalyst, and auxiliaries was deemed to be very low. The raw material cost was estimated as 85.8 percent of the total yearly operating costs.

Production of Hydrogen

Hydrogen is a clean fuel and feedstock to the energy and industrial chemicals industries.[227] An interesting process produces hydrogen, the aqueous-phase reforming of glycerol.[228] In their study, Liu and others focused on ethylene glycol (EG), a chemical found in the cooling system of most vehicles, and on glycerol, a by-product of the hydrolysis of fats and oils to make biodiesel. Both EG and glycerol could be reformed to hydrogen and carbon dioxide at 240 °C. Hydrogen yields from reforming EG and glycerol were 82% and 70%, respectively. The benefit of this operation is the conversion of glycerol to a more valuable product, hydrogen that can be used as a fuel in fuel cells.[229]

223. Knothe G. (2008), "«Designer» Biodiesel: Optimizing Fatty Ester Composition to Improve Fuel Properties", *Energy & Fuels*, 22: 1358–1364; *Knothe G. (2005), "Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters", Fuel* Processing Technology, 86: 1059–1070.

224. Wang P. S., M. E. Tat, J. Van Gerpen (2005), "The production of fatty acid isopropyl esters and their use as a diesel engine fuel", *Journal of the American Oil Chemists' Society*, 82 (11): 845-849; Miller S. J. (2011), US Pat. 20110239529, *Biodiesels useful for improving cloud point* (06-Oct-2011); Lee I., L. A. Johnson, E. G. Hammond (1995), "Use of branched-chain esters to reduce the crystallization temperature of biodiesel", *Journal of the American Oil Chemists' Society,* 72 (10): 1155–1160.

225. Ryan C. C. (2008), "Additive lowers tallow-based biodiesel cloud point", *Biodiesel Magazine*, October 14, 2008 (www.biodieselmagazine.com).

226. Nelson R. G., S.A. Howell, J. Weber (1994), *Potential Feedstock Supply and Costs for Biodiesel Production*, presented at the sixth national bioenergy conference in Nevada, October 2-8, (www.biodiesel.org/resources/ reportsdatabase/reports/gen/19941006_gen-290).

227. Gupta R. B. (2009), *Hydrogen Fuel: Production, Transport, and Storage*, CRC Press, Florida (USA).

228. Liu B., Y. Zhang, J. W. Tierney, I. Wender (2005), *Hydrogen by Catalytic Reforming of Glycols*, in "C1 Chemistry for the Production of Ultra-Clean Liquid Transportation Fuels and Hydrogen", Semi-annual six-month report. Research conducted October 1, 2004-March 31, Prepared by the Consortium for Fossil Fuel Science, Gerald P. Huffman, Director CFFS/University of Kentucky, Lexington, KY, pp. 75-77.

229. Fuel cells are electrochemical devices that convert chemical energy directly to electricity. They offer a significant inherent advantage over typical internal combustion engine where efficiency is lost due to the conversion of stored chemical energy first to heat energy, then to mechanical energy, and finally to electricity. Fuel cells are not subject to Rankine/Carnot cycle efficiency limitations and are energy carriers, like batteries. In theory, a fuel cell can continue to produce power indefinitely if a fuel stream such as hydrogen is constantly provided.

4.6 Glands and organs

Animal glands are traditionally used as medicine in many Western and Eastern countries, including China, India, South Korea, New Zealand and Japan.

The more exploited are liver, pituitary, thyroid, pancreas, stomach, adrenal glands, kidney, spleen, lungs, thymus, thyroid, and ovary. Different animals have different glands that are important. Their function also depends on the species, sex and age of the animal. They are collected only from healthy animals.

a) Adrenal glands

Mammals adrenal glands are endocrine glands that sit at the top of the kidneys. They regulate the response to stress by secreting hormones and are divided into two parts: the inner portion (medulla) which secretes at least 20 steroids that are essential for maintaining life (among these it secretes are epinephrine and norepinephrine); and the outer portion (the cortex) which manufactures the hormones cortisone and aldosterone.

Adrenal extract is a chemical made from the inner portion of the adrenal glands of slaughtered cows, pigs, and sheep. It is a trisubstituted benzene and is used as a heart stimulant. By mouth, adrenal extract is used for low adrenal function, fatigue, asthma, stress, severe allergies, lowered resistance to illness, certain skin conditions such as eczema and psoriasis, and rheumatoid arthritis. Recent findings[230] show its role in the treatment of anaphylaxis,[231] croup,[232] bronchiolitis,[233] and as an adjunct to local anesthesia.[234]

Steriods from the cortex of the adrenal gland regulate the body's utilization of nutrients such as fat, carbohydrates, water and minerals. Steriods extracted from cattle, pigs or sheep are used as anti-inflammatory agents and for the treatment of shock and asthma. One such steroid, corticosterone, derived from bovine adrenals, is extracted directly from the cortex of the adrenal glands.

Adrenalin, without the "e", was originally used as a trademark for a product made by an American pharmaceutical firm Parke, Davis & C[235]. The usage of the word, with or without the "e", however, was not very consistent; thus the term's use seems interchangeable.

Epinephrine and nor-epinephrine, extracted from the medulla of cattle, pigs, and sheep, are glande extracts used to stop hemorrhaging, stimulate heart action and overcome shock.

230. See also: Walker D. M. (2009), *Update on epinephrine (adrenaline) for pediatric emergencies*, Lippincott Williams & Wilkins, Inc., Wolters Kluwer N.V.

231. Garvey L. H., B. Belhage, M. Krøigaard, B. Husum, Hans-Jørgen Malling, H. Mosbech (2011), "Treatment with epinephrine (adrenaline) in suspected anaphylaxis during anesthesia in Denmark", *Anesthesiology*, 115(1): 11-6.

232. Racemic epinephrine has historically been used for the treatment of croup. See: Thomas L. P., L. R. Friedland (1998), "The cost-effective use of nebulized racemic adrenaline in the treatment of croup", *American Journal of Emergency Medicine,* 16 (1): 87–89.

233. Menon K., T. Sutcliffe, T. P. Klassen (1995), "A randomized trial comparing the efficacy of epinephrine with salbutamol in the treatment of acute bronchiolitis", *The Journal of Pediatrics*, 126(6): 1004-1007.

234. Wong J. K. (2001), "Adjuncts to Local Anesthesia: Separating Fact from Fiction", *Journal of the Canadian Dental Association*, 67: 391-7.

235. The Parke-Davis Research Laboratory is an American National Historic Landmark; the surrounding Parke-Davis and Company Pharmaceutical Company Plant is on the National Register of Historic Places. Parke-Davis was acquired by Warner-Lambert in 1970, which in turn was bought by Pfizer in 2000.

The molecular formula for epinephrine is $C_9H_{13}NO_3$. Epinephrine is derived from tyrosine, an aminoacid. Epinephrine is sometimes referred to as a catecholamine as it contains the catechol moiety. This is a part of the molecule that contains the group $C_6H_4(OH)_2$.

b) Kidney

Animal kidneys (from cattles, pigs, or sheeps) after having been removed from the fatty capsule which holds them in place, may be cooked whole or in slices. Kidney is generally broiled, grilled, or braised.

For transplantation

Kidney was one of the first organs to be used for transplantation. Historically, kidney transplantation is thought to have originated early in the twentieth century with several attempts of xenografting and experimental works on vascular sutures.[236]

The first successful experimental kidney transplant was performed at the Vienna Medical School in Austria in 1902. The surgeon Ullmann[237] reported the first case of renal autotransplantation performed in the neck of a dog. In 1906, M. Jaboulay,[238] in Lyon, used kidneys from pigs and goats to attempt xenotransplantation in a human patient. According to records the first kidney transplant experiments were performed in France in 1909 on humans using animal kidneys.[239] In this experiment a surgeon inserted slices of rabbit kidney into a child suffering from kidney failure. Although "the immediate results were excellent" the child died about 2 weeks later. In 1909, Ernst Unger used en-bloc Macaccus kidneys in humans that rapidly failed, due to an unknown hyperacute vascular rejection.[240] Scientists of the time believed kidney xenotransplants were possible, but their success was limited by unknown "biochemical barriers," which prevented long-term kidney survival.

The site of the first successful human-to-human kidney transplant was at the Peter Bent Brigham Hospital in Boston, Massachusetts, where, in 1954, Joseph E. Murray transplanted

236. Küss R., P. Bourget (1992), *Une histoire illustrée de la greffe d'organes. La grande aventure du siècle*, Laboratoires Sandoz, Rueil-Malmaison (France); Taniguchi S., D. K. Cooper (1997), "Clinical xenotransplantation: past, present and future", *Annals of The Royal College of Surgeons of England*, 79 (1): 13–19; Cooper D. K. C. (2012), "A brief history of cross-species organ transplantation", Baylor University Medical Center Proceedings, 25 (1): 49–57.

237. Emerich Ullmann was Chief Surgeon at Spital der Baumherigen Schwetern, Germany.

238. Schlich T. (2011), "The origins of organ transplantation", *The Lancet*, 378 (9800): 1372 – 1373.

239. Princeteau M. J. (1905), "Greffe renale", *Journal de Medicine de Bordeaux*, 26: 549; Reemlsma K. (1969), "Renal heterotransplantation from nonhuman primates to man", *Annals of the New York Academy of Sciences*, 162: 412-8; Deschamps J.-Yves, F. A. Roux, P. Saï, E. Gouin (2005), "History of xenotransplantation", *Xenotransplantation*, 12 (2): 91–109.

240. For more notices see: Nagy J. (1999), "A note on the early history of renal transplantation: Emerich (Imre) Ullmann", *American Journal of Nephrology*, 19 (2): 346-9; Jorge O. (2011), A. Jason (Edtrs.), *Understanding the Complexities of Kidney Transplantation*, InTech, Rijeka, Croatia (available at www.intechopen.com); Brent L. (1997), *A history of transplantation immunology*, Academic Press, San Diego, (CA-USA); Doyle A. M., R. I. Lechler, L. A. Turka (2004), "Organ Transplantation: Halfway through the First Century", *Journal of the American Society of Nephrology*, 15 (12): 2965-2971; Schlich T. (2010), *The origins of organ transplantation: surgery and laboratory science, 1880s—1930s,* University of Rochester Press, Rochester, (NY-USA).

a kidney that was donated by the patient's twin.[241] Many other efforts followed but all failed with short survival of the affected,[242] except one. The only success was the nine-month survival of a chimpanzee kidney transplanted into a human by Reemtsma and colleagues.[243] No one knows why this survival occurred, in the 1960s there were no powerful immunosuppressive agents, and no known immunological or genetic manipulations of donor or recipient.

Xenotransplantation was seen by some as an opportunity and by others as a danger. It could help overcome the shortage of organs from human donors, but it raised many questions about safety, ethics and human nature.[244] As a consequence many researchers settled on the use of pigs as potential source animals for xenotransplantation.[245] In most recent years, researchers are experimenting with producing organs from genetically modified animals. With the advent of genetic engineering and cloning technologies, pigs are currently available with a number of different manipulations that protect their tissues from the human immune response. The use of genetic engineering has resulted in significant improvement in survival time for a pig organ in a non-human primate receiving immunosuppression.[246]

241. Morris P. (2013), "Joseph E. Murray (1919–2012)", *Nature*, 493 (7431): 164; Guild W. R., J. H. Harrison, J. P. Merrill, J. Murray (1955), "Successful homotransplantation of the kidney in an identical twin", *Transactions of the American Clinical and Climatological Association*, 67: 167–173.

242. Mohacsi P. J., J. F. Thompson, S. Quine (1998), "Attitudes to xenotransplantation: scientific enthusiasm, assumptions and evidence", *Annals of Transplantation*, 3 (2): 38–45.

243. Reemtsma K., B. H. McCracken, J. U. Schlegel, M. A. Pearl, C. W. Pearce, C. V. DeWitt, P. E. Smith, R. L. Hewitt, R. L. Flinner, O. Jr Creech (1964), "Renal hetero xenotransplantation in man", *Annals of Surgery*, 160: 384–410.

244. Daar A. S. (1999), "Animal-to-human organ transplants – a solution or a new problem?", *Bulletin of the World Health Organization*, 77 (1): 54-61.

245. Cooper D. K. C., Y. Ye, J. L. L. Rolf, N. Zuhdi (1991), *The Pig as Potential Organ Donor for Man*, in "Xenotransplantation. The Transplantation of Organs and Tissues Between Species", D. K. C. Cooper, E. Kemp, K. Reemtsma, D. J. G. White (Edts.), 1st ed., Springer-Verlag, Berlin, pp. 481–500; Hammer C., R. Linke, F. Wagner, M. Diefenbeck (1998), "Organs from animals for man", *International Archives of Allergy and Immunology*, 116: 5–21.

246. Cozzi E., D. J. G. White (1995), "The generation of transgenic pigs as potential organ donors for humans", *Nature Medicine*, 1: 964 - 966; Lin S. S., B. C. Weidner, G. W. Byrne, L. E. Diamond, J. H. Lawson, C. W. Hoopes, L. J. Daniels, C. W. Daggett, W. Parker, R. C. Harland, R. D. Davis, R. R. Bollinger, J. S. Logan, J. L. Platt (1998), "The role of antibodies in acute vascular rejection of pig-to-primate cardiac transplants", *The Journal of Clinical Investigation*, 101 (8): 1745–1756; Cozzi E., F. Bhatti, J. R. Bradley, G. Chavez, P. J. Friend, M. Goddard, D. Ostlie, M. Schmoeckel, K. G. C. Smith, S. Thiru, C. Vial, J. Wallwork, D. J. G. White, A. Zaidi (2000), "Long-term survival of non-human primates receiving life-supporting transgenic porcine kidney xenografts", *Transplantation*, 70 (1): 15–21; Vial C. M., D. J. Ostlie, F. N. Bhatti, E. Cozzi, M. Goddard, G. P. Chavez, J. Wallwork, D. J. White, J. J. Dunning (2000), "Life supporting function for over one month of a transgenic porcine heart in a baboon", *The Journal of Heart and Lung Transplantion*, 19 (2): 224–229; Loss M., B. Vangerow, J. Schmidtko, R. Kunz, A. Jalali, H. Arends, M. Przemeck, H. Rückholt, M. Leuwer, F. J. Kaup, S. Rensing, E. Cozzi, D. J. White, J. Klempnauer, M. Winkler (2000), "Acute vascular rejection is associated with systemic complement activation in a pig-to-primate kidney xenograft model", *Xenotransplantation*, 7 (3): 186–96; Bhatti F. N., M. Schmoeckel, A. Zaidi, E. Cozzi, G. Chavez, M. Goddard, J. J. Dunning, J. Wallwork, D. J. White (1999), "Three-month survival of HDAF transgenic pig hearts transplanted into primates", *Transplantation Proceedings*, 31 (1-2): 958-66; Diamond L. E., C. M. Quinn, M. J. Martin, J. Lawson, J. L. Platt, J. S. Logan (2001), "A human CD46 transgenic pig model system for the study of discordant xenotransplantation", *Transplantation*, 71 (1): 132-42;

The first batch of genetically modified pigs was born in the second half of 2011. Genetic engineering experts at the Nanjing Medical University (in eastern China's Nanjing) recently announced that these pigs are expected to provide suitable organs for transplant into human bodies and to ease the nation's shortage of transplant organs. Researchers at that Medical University said that pig-to-human heterografts are expected to undergo clinical trials within two to three years and that the exact time needed depends on the type of organs concerned.

c) Liver

The liver is the largest gland in animals. Liver from lamb, veal calves, pigs and young cattle are the most widely used edible organ. Collected only from healthy animals, liver is used in many processed meats, such as liver sausage and liver paste, especially in the United States and Europe. In theses countries consumers generally prefer livers from pigs because it has a lighter flavor and texture.

Liver extract

The liver of mature cattle weighs about 5 kg, while that of a pig weighs approximately 1.4 kg.[247] For use as raw material in the pharmaceutical industry, liver extract[248] is produced by mixing raw ground liver from pigs and cattle with slightly acidified hot water. The stock is concentrated in a vacuum to make a paste.[249] That paste has longevity as a source of vitamin B_{12}, as a nutritional supplement used to treat various types of anemia, for production of red blood cells, as a homeopathic medicine, and as a micro nutrient.

Heparin can be extracted from the liver and used as an anticoagulant to prolong the clotting time of blood, to thin the blood, and to prevent blood clotting during surgery and in organ transplants.

For transplantation

The first successful human liver transplant was performed by Starzl and colleagues at the the University of Colorado in 1967. The recipient lived for about a year before dying from a recurrence of liver cancer. In the 1990s, Starzl and colleagues[250] transplanted baboon livers into one patient. This patient survived for twenty-six days. The operation marks the first known baboon-to-human liver transplant in the world. The baboon was selected as a donor because it is not an endangered species, and can be bred safely and easily in captivity. A baboon pathogen (cytomegalovirus) was apparently transferred to the patient, even

Chang A. T., J. L. Platt (2009), "The role of antibodies in transplantation", *Transplantation Reviews,* 23 (4): 191–198.

247. To know more about see: Boxenbaum H. (1980), "Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin", *Journal of Pharmacokinetics and Biopharmaceutics*, 8 (2): 165-176.

248. Liver extract is a dietary supplement made from animal liver, often pig or cow, and is commonly sold either as a freeze-dried powder or a concentrated liquid. It contains vitamin B_{12}, folic acid and iron.

249. Fenger F., *Production of liver extract*, United States Patent 2045266.

250. Starzl T. E., J. J. Fung, A. G. Tzakis, S. Todo, A. J. Demetris, I. R. Marino, H. Doyle, A. Zeevi, V. Warty, M. Michaels, S. Kusne, W. A. Rudert, M. Trucco (1993), "Baboon-to-human liver transplantation", *The Lancet*, 341 (8837): 65-71.

though this did not result in a disease process.[251] However, in the patient there was evidence of an adequately functioning liver mass, sufficient to sustain life. The baboon liver led to the presence of baboon proteins synthesized by the liver; in some cases those proteins assumed the blood levels characteristic of the baboon and not of the human.

On June 28, 1992, Starzl complete an 11-hour transplant operation of a baboon liver into a 35-year-old male, but the patient died after 71 days as doctors tried to wean him from a ventilator. From 1966 to 1973 Tom Starzl transplanted three livers from chimpanzees to children. All died within two weeks.[252] After the last transplant, protesters picketed Starlz's house calling him "Tom FrankenStarzl".

In the meantime, scientists continue to investigate pig's liver. In 1993 Leonard Makowka put a pig liver into a human and failed.[253] Currently, future liver xenotransplantation hopes rest on animals, particularly genetically modified pigs. Pigs could provide an alternative source of tissue and cells, but the immunological challenges and other barriers associated with xenotransplantation need to be overcome. Transplantation of organs from genetically modified pigs into non-human primates is now not substantially limited by hyperacute, or acute antibody-mediated, or cellular rejection; other issues have become more prominent, such as development of thrombotic microangiopathy in the graft or systemic consumptive coagulopathy in the recipient.[254]

Genetically modified pig liver[255] is used for ex vivo perfusion in the treatment of fulminant liver failure.

d) Pancreas

Pancreatic tissue is present in all vertebrate species, and its form and arrangement vary widely. In teleosts, and a few other species (such as rabbits), there is no discrete pancreas at all, with pancreatic tissue being diffusely distributed across the mesentery and even within other nearby organs, such as the liver or spleen.

Juices for metabolic use

The pancreas, as do other glands of the animal body, produces juices for metabolic use that help break down food, and it produces hormones that help control blood sugar levels. Juices are a source of raw material. Among these substances are insulin, glucagon,

251. Michaels M. G., F. J. Jenkins, K. St George, M. A. Nalesnik, T. E. Starzl, C. R. Rinaldo Jr. (2001), "Detection of infectious baboon cytomegalovirus after baboon-to-human liver xenotransplantation", *The Journal of Virology*, 75 (6): 2825–8.

252. After Tom Starlz' failed xeno transplants public and professional attitudes hardened saying that humans shouldn't be used for virtual experiments until further progress was made on reducing immune reaction to animal organs.

253. Cooper D. K. C., R. P. Lanza (2000), *Xeno: The Promise of Transplanting Animal Organs Into Humans*, Oxford University Press, N. Y., p. 41.

254. Ekser B., M. Ezzelarab, H. Hara, D. J. van der Windt, M. Wijkstrom, R. Bottino, M. Trucco, D. K. C. Cooper (2001), "Clinical xenotransplantation: the next medical revolution?", *Transplantation*, 71 (1): 132-42; Elliott, R. B. (2011), "Towards xenotransplantation of pig islets in the clinic", *Curr Opin Organ Transplant*, 16 (2): 195-200..

255. Diamond L. E., C. M. Quinn, M. J. Martin, J. Lawson, J. L. Platt, J. S. Logan (2001), "A human CD46 transgenic pig model system for the study of discordant xenotransplantation", ibid., 132-42.

pancreatin, somatostatin, pancreatic polypeptide etc. Major products such as pepsin, rennin and other digestive enzymes, lipase and trypsin enzymes extracted from the pancreas are all medically significant products. Chymotrypsin and trypsin are used to improve healing after surgery or injury.

Insulin

Insulin is a hormone consisting of two linked polypeptide chains, which regulates sugar metabolism and is used in the treatment of diabetes. The structure of human insulin is similar but not identical to the bovine and porcine insulin produced by the Meat Industry.

The attempts to produce insulin outside the human body, dates back to 1921 when Banting and Best,[256] and Macleod,[257] successfully purified insulin from a dog's pancreas.

Though insulin has been referenced as one of the prime pharmaceutical products derived from ABPs, it is now synthesized by other procedures.[258] This is true for a number of other pharmaceuticals, but reliance on the natural production and extraction is still an important source of medical treatment and prevention compounds.

Pig insulin has been used to treat diabetes in humans until the 1980s, making the pigs potentially good islet donors for humans with the disease. Insulin from pigs was routinely given to patients with diabetes, until DNA technology enabled pharmaceutical companies to manufacture human insulin. In a recent study,[259] the researchers transplanted clusters of embryonic pig pancreatic cells into 10 diabetic rats that could not produce insulin on their own and that had very high glucose levels. The cells were retrieved from the pig embryos early in their development, which was believed to make them both "invisible" to the rats' immune system and also not to induce a state of immune tolerance. Those who receive human islet transplants must take anti-rejection drugs for the rest of their lives, so essentially they are trading daily insulin shots for immune-suppression drugs, which carry their own risks. Pig cells could overcome the shortage of human islets available from deceased donors and the need for transplant patients to take anti-rejection drugs for life.

Since about 20 years are ago, immune protective devices containing pancreatic islets designed to treat insulin-dependent diabetes mellitus have been studied and tested for their ability to provide glycemic control without immunosuppression.[260] The device consists of

256. Best C. H., D. A. Scott (1923), "The Preparation of Insulin", *The Journal of Biological Chemistry*, 57 (3): 709-723.

257. Simoni R. D., R. L. Hill, M. Vaughan (2002), "The Discovery of Insulin: the Work of Frederick Banting and Charles Best", *The Journal of Biological Chemistry*, 277 (26): 31-32.

258. In 1982, the Eli Lilly Corporation successfully produced a human insulin, that became the first approved genetically engineered pharmaceutical product. Human insulin is grown in the lab inside a common bacteria, Escherichia coli. Though it is by far the most widely used type of bacterium, yeast can also be used as a substitute.

259. Rogers and others, at Washington University School of Medicine, have alleviated the diabetic in rats using transplants from both embryonic and adult pigs. The rats adopted the pig transplants as their own and produced enough insulin to control their blood sugar, without the need for anti-rejection drugs (See: Rogers S. A., T. Mohanakumar, H. Liapis, M. R. Hammerman (2010), "Engraftment of cells from porcine islets of Langerhans and normalization of glucose tolerance following transplantation of pig pancreatic primordia in non-immune suppressed diabetic rats", *American Journal of Pathology*, 177 (2): 854–864).

260. Maki T., C. S. Ubhi, H. Sanchez-Farpon, S. J. Sullivan, K. Borland, T. E. Muller, B. A. Solomon,

a chamber through which passes a copolymer membrane connected to standard vascular grafts. Islets are placed inside the chamber but are outside of the blood stream. Further research has been conducted using rats.[261] In other studies[262] pig pancreatic islets (encapsulated) have been used for the treatment of diabetic dogs. Preliminary obtained data suggests that pig islet microencapsulation achieved metabolic control in type I diabetic dogs without the risk of immunosuppression using one or two procedures per year.

Glucagon

Glucagon is a 29-amino acid polypeptide,[263] named in 1923, from the Greek γλυκός.[264] Unlike insulin, glucagon extracted from the pancreas is used to increase blood sugar, to lower blood sugar caused by alcoholism, and to treat insulin overdoses.

Bovine glucagon[265] has the same amino acid composition as human[266] and porcine glucagon. Glucagon can be produced synthetically[267] but as the structures of bovine, porcine and human glucagon are identical, that synthetic has no advantage over natural glucagon.

The process for recovering glucagon from pancreas glands has been the subject of numerous patents.[268]

Pancreatin

Pancreatin is a mixture of digestive enzymes consisting primarily of amylase, lipase and protease. A mixture of those pancreatic juice enzymes, extracted from animals such as cattle

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W. L. Chick, A. P. Monaco (1991), "Successful treatment of diabetes with the biohybrid artificial pancreas in dogs", *Transplantation*, 51 (1): 43-51; Maki T., C. J. Mullon, B. A. Solomon, A. P. Monaco (1995), "Novel delivery of pancreatic islet cells to treat insulin-dependent diabetes mellitus", *Clinical Pharmacokinetics*, 28 (6): 471-82.

261. See for example: Rogers S. A., F. Chen, M. Talcott, M. R. Hammerman (2004), "Islet cell engraftment and control of diabetes in rats after transplantation of pig pancreatic anlagen," *American Journal of Physiology*, 286 (4): E502–E509.

262. See: Abalovich A. G., M. C. Bacqué, D. Grana, J. Milei (2009), "Pig pancreatic islet transplantation into spontaneously diabetic dogs", *Transplantation Proceedings*, 41 (1): 328-30.

263. Bromer W. W., L. G. Sinn, A. Staub, O. K. Behrens (1957), "The amino acid sequence of glucagon", *Diabetes*, 6 (3): 234-8.

264. To know more about see: Kieffer T. J., J. F. Habener (1999), "The Glucagon-Like Peptides", *Endocrine Reviews*, 20 (6): 876-913.

265. Bromer W. W., M. E. Boucher, J. E. Koffenberger (1971), "Amino acid sequence of bovine glucagon", *Journal of Biological Chemistry*, 246 (9): 2822-2827.

266. Thomsen J., K. Kristiansen, K. Brunfeldt, F. Sudky (1971), "The amino acid sequence of human glucagon", *FEBS Letters*, 21 (3): 315-319.

267. Lundt B. F., F. C. Groenvald, N. L. Johansen, J. Markussen (1979), "Synthesis of glucagon", *Research Disclosure*, 181: 246-247.

268. Among others see: Maskalick D. G., M. T. Anderson - US Patent 4,617,376, 1986; Maskalick D. G., M. T. Anderson - EP Patent 0,207,727, 1987; Smith M. R. - US Patent 3,715,345, 1973; Stilz J. G., R. L. Jackson - US Patent 4,033,941, 1977; Moody A. J., L Thim, K. D. Jorgensen - US Patent 4,405,608, 1983; Ditte Riber (Frederiksberg, DK), Eddi Meier (Vaerlose, DK), Trine Skovlund Ryge (Frederikssund, DK), Jens Rosengren Daugaard (Virum, DK) - Patent application number: 20100204105, 2010; Drucker D. J. (Toronto, CA), A. E. Crivici (San Diego, CA, US), M. Sumner-Smith (Bolton, CA) - Patent application number: 20110009320, 2011.

or hogs is used as a digestive aid. Supplemental pancreatic enzyme preparations are provided to patients with conditions of pancreatic exocrine deficiencies such as chronic pancreatitis and cystic fibrosis.[269] Pancreatin is also used for improving digestion of fatty foods, to assist specifically with the digestion of carbohydrates, lipids and proteins and it is used in pancreatitis control.[270]

As capsules, the enzyme found on the open market might help people suffering from:

- Cystic fibrosis
- Different forms of cancer
- Heart disease
- Viruses

Pancreatic enzyme preparations, generically called pancreatin, are not alike. Rather, they present a broad variety of pancreatin composition.[271]

Pancreatin is produced by activation, extraction, precipitation, degreasing, drying, and crushing of milled pig pancreas. Porcine pancreas is preferred for the industrial manufacture of Pancreatin for the following reasons: it is commercially available in substantial quantities; it contains high Protease, Amylase and Lipase activity (provided the glands were frozen immediately after slaughtering); and the composition is similar to that of human pancreas. Some methods of preparation are covered by patents. A dry pancreatin preparation[272] is produced from a still moist pancreatin mass, which is obtained after extraction with a solvent or solvent mixture. Before the solvents are finally removed, it is then treated,, for a short time in a vacuum cutter until the mass has been divided and shaped into small spherical particles, and then it is finally dried.

e) Pituitary glands

The pituitary gland (or hypophysis), known as the "master gland" of the endocrine system, is located at the base of the brain and produces several species of hormones that help in the control of many processes.[273] Among the most important hormones, there are the Growth-promoting Hormone (GH) (excess of GH can lead to gigantism), the thyroid stimulating hormone (TSH), the mammary stimulating hormone and the adrenal-cortex stimulating hormone (ACTH). Other types of hormones, secreted from the pituitary gland, are responsible for controlling blood pressure, breast milk production, conversion of food into energy, temperature regulation, sex organ functions (in both males and females), and pain relief, etc.

Major hormonal changes emerge during pregnancy. The pituitary gland is one of the organs most affected with altered anatomy and physiology. Due to physiological changes in

269. Ferrone M., M. Raimondo, J. S. Scolapio (2007), "Pancreatic enzyme pharmacotherapy", *Pharmacotherapy*, 27 (6): 910-20.

270. Read more at http://www.drugs.com/cdi/pancreatin.html.

271. Löhr J. M , F. M. Hummel, K. T. Pirilis, G. Steinkamp, A. Körner, F. Henniges (2009), "Properties of different pancreatin preparations used in pancreatic exocrine insufficiency", *European Journal of Gastroenterology & Hepatology*, 21 (9): 1024-1031.

272. Atzl G., F. Langer, H. Polleres - US Patent 5,861,177, 1999; Wilson Pharmaceutical & Chemical Corporation - 3,956,483, 1971; Kali-Chemie Pharma GmbH – US Patent 4,019,958, 1977; CAN Technologies, Inc. US Patent 7,153,504 – 2006.

273. Melmed S. (2011), *The pituitary*, Academic Press, third edition, London; Charkravarthy R. Kannan (1987), *The pituitary gland*, Plenum Medical Book Co, New York.

the pituitary and target hormone levels, binding globulins, and placental hormones, hormonal evaluation becomes more complex in pregnant women.[274]

ACTH, the main hormone extracted from the pituitary, is used as a treatment for rheumatism, arthritis, eye inflammation and multiple myeloma.

The induction of amphibians ovulationwith the use of hormones has been used since the early 20th century. Some amphibians have never reproduced in captivity without hormones and by using hormonal induction many species can be reproduced at will.[275] Pituitary hormones have been very successful in encouraging ovulation in some organisms when purified hormones did not succeed. But there are disadvantages: they may not be commercially available in all countries and have the potential to transmit disease.

Anyhow, artificially injected hormones in animals can be profitable for the farmers as they help the animals to gain weight as well as increase the amount of milk produced.[276] Various hormones such as "Bovine Growth Hormone" (BGH) estrogen can be artificially introduced into the cow pituitary glands to increase the production of milk. There are questions about the possible side effects on the human body after consuming the hormones in meat and dairy products.[277] The practice of injecting hormones in cattle and poultry is very common, and down the line can be harmful for humans and animals, as well as the environment, and might cause side effects like mastitis.[278]

BGH, also called "bovine somatotropin" (BST), produced in the pituitary glands of dairy cows, is a naturally occurring protein hormone in milk, which stimulates the liver to produce insulin-like growth factor-I.[279] In the early stages of a calf's development, BST acts as a growth hormone. During lactation, it serves to mobilize body fat for use as energy and diverts feed energy more toward milk production than toward tissue synthesis. For these reasons a large number of cattle in the U.S. and in the E. U. are supposedly being fed BGH in order to increase milk production.

In the past GH was extracted from human pituitary glands and given to deficient children and cattle. Soon with the increase in consumption, the quantity of GH produced from pituitary glands was insufficient to meet the demand. As a consequence biotechnology research received a boost to produce hormones using genetically engineered bacteria. The

274. Karaca Z., F. Tanriverdi, K. Unluhizarci, F. Kelestimur (2010), "Pregnancy and pituitary disorders", *European Journal of Endocrinology*, 162 (3): 453-475.

275. One of the great advantages of hormonal induction is the production of oocytes without fertilisation by males. Several classes of compounds are used as hormones: compounds such as pimozide, pituitary extracts, etc.

276. Today, the cattle are often raised by using artificial hormones to increase the growth rate as well as the body mass. Artificially induced hormones in animals can increase the dairy as well as meat production. Read more at: http://www.buzzle.com/articles/hormones-in-food.html.

277. After thorough research, the SCVPH (Sub-Committee on Veterinary Public Health) came to the conclusion that "no acceptable daily intake could be established for any of these hormones" and those who eat food products having these hormonal residues are at a great risk of severe hormonal imbalance as well as various types of cancer.

278. Udder infection causing pus that can later get into the milk that we consume. As a consequence, they are given some type of antibiotics that cause more trouble.

279. The structure of human somatotropin differs from BGH, and the latter seems not to be biologically active in humans. See: Parodi P. W. (2005), "Dairy product consumption and the risk of breast cancer", *Journal of the American College of Nutrition*, 24 (6): 556S–568S.

genes responsible for production of BST in cattle were identified in bovine tissue cells; they cause the pituitary cells to produce the biological product BST. These genes were isolated and inserted into specific bacteria as part of a plasmid. As these altered bacteria replicated, the new genes are also replicated and passed along to all new bacteria that essentially become little "manufacturing plants" able to produce BST in large quantities. In milk the synthetic form of BST cannot be distinguished from natural form. Somatrem and Somatropin are man-made versions of human growth hormone.[280]

GH is now produced synthetically and given to both children and adults for a variety of reasons,[281] but GH therapy is a focus of social and ethical controversies.

f) Spleen

"The spleen has been considered a mysterious organ since classical times. For many years after its existence became known, it appeared to have no function. Now we know this to be far from the truth, yet many individuals survive perfectly well in the absence of a functioning spleen, following surgery or as a result of diseases causing hyposplenism".[282]

Spleen, as it is well known, is a large, highly vascular lymphoid organ present in virtually all vertebrate animals. It serves to store blood, disintegrate old blood cells, filter foreign substances from the blood, and produce lymphocytes. It also holds a reserve of blood that can be released into the circulatory system to meet a sudden demand, such as bleeding (hemorrhage).

Spleen's main function is to act as a filter for the blood. It recognizes and removes old, malformed, or damaged red blood cells. When blood flows into the spleen, the red blood cells must pass through a labyrinth of narrow passages. Healthy blood cells can pass through the spleen and continue to circulate throughout the bloodstream, while blood cells that are not able to pass will be broken down in the spleen by macrophages. Useful components from the old cells, such as iron,[283] will be saved Unfortunately no one completely understands the spleen's functions.[284]

Another function of the spleen is the accumulation of blood. Depending on the amount accumulated, the spleen is able to get wider or narrower, to meet the needs of the bodies. When it is expanded it can hold a great reserve of blood in order to respond to further requests for blood (due to a trauma, during transfusions, etc.).

The spleen also plays an important role in the immune system, which allows you to fight infection. When it detects faulty red blood cells, the spleen identifies and pick out enemy or unwelcome micro-organisms (bacteria, viruses, etc.) in the blood.

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280. Bramnert M., M.Segerlantz, E. Laurila, J. R. Daugaard, P. Manhem, L. Groop (2003), "Growth hormone replacement therapy induces insulin resistance by activating the glucose-fatty acid cycle", *The Journal of Clinical Endocrinology & Metabolism*, 88 (4): 1455-1463.

281. Man-made growth hormone may be used in children who have failure to grow normally. Among the reasons there are the inability to produce enough growth hormone, kidney disease, Prader-Willi Syndrome, and Turner's syndrome. Growth hormone is also used in adults to treat growth failure and to treat weight loss caused by acquired immunodeficiency syndrome (AIDS).

282. Wilkins B. S. (2002), "Historical Review", 2002 Blackwell Science Ltd, *British Journal of Haematology*, 117 (2): 265–274.

283. Spleen stores iron in the form of ferritin or bilirubin, and returns the iron to the bone marrow.

284. Wilkins B. S. (2002), "Historical Review", ibid.: 265.

The spleen continues to produce red blood cells throughout life in most vertebrates. Many mammals possess tiny spleen-like structures known as haemal nodes throughout the body, which, it is presumed, have the same function as the spleen proper.

In addition to food

Spleen seems also to be a good food. For instance, spleen is minced and used in pies and as a flavoring in the United Kingdom, and as an ingredient in processed meat in the United States. To be safely eaten it must, of course, be perfectly healthy.[285] But everyone knows that it is subject to various diseases. One thinks of hog cholera, tuberculosis, charbon, and other diseases of the same group. If it is not perfectly fresh, the large number of blood-corpuscles in the spleen pulp renders it peculiarly prone to decomposition. When cooked, it keeps better.

Its softness and the necessity of eating spleen only when it is quite fresh are reasons why it is unmarketable in their natural state and can only be obtained at the slaughter-houses.

Ferritin

In addition to food, spleen can be used to obtain ferritin from horse (equine) and cattle (bovine) that are used in the treatment of iron-deficiency anemia. "Spleen extract" is given "as replacement therapy" in cases where the spleen has been surgically removed and or isn't working properly.

For stimulating the immune system

Spleen extract is also used for treating "autoimmune" diseases such as celiac disease, systemic lupus erythematous (SLE), dermatitis herpetiformis, and rheumatoid arthritis. Preliminary studies indicate that spleen extract may stimulate the immune system in conditions such as HIV/AIDS, leukemia, leprosy, Crohn's disease, and sickle cell disease. Some even use spleen extract for glomerulonephritis, thrombocytopenia, ulcerative colitis, and a blood vessel condition called vasculitis.

Some concern has been raised about the safety of spleen extract, as it is made of animal spleens, which may be infected with some diseases: prion, bovine spongiform encephalitis, etc.

g) Thymus

The thymus (its name comes from the Greek word θυμός and is called thymus because its shape resembles that of a thyme leaf) is a specialized organ of the immune system. The thymus "instructs" T-lymphocytes (T cells), which are critical cells of the adaptive immune system. Each T cell attacks a foreign substance that it identifies with its receptor.[286] Thymus extracts could have substances that influence the immune system, but it is very difficult to exactly know what effect these extracts have on the immune system. The thymus seems to be much less important in adults as it ceases to function after childhood.

The thymus is composed of two identical lobes and is located anatomically located in the neck or chest of most vertebrate animals. In humans it is located in the anterior superior

285. Williams E. T. (1906), *The Edibility of Animal Spleens, Reprinted from "American Medicine"*, Vol. XI, No. 6, The Library of Congress, Washington DC.

286. Miller J. F. (2002). "The discovery of thymus function and of thymus-derived lymphocytes", *Immunological Reviews*, 185 (1): 7–14.

mediastinum, which is in front of the heart and behind the sternum. It is especially important in newborn babies: without a thymus a baby's immune system collapses and the baby will die. Histologically, the thymus can be divided into a central medulla and a peripheral cortex that is surrounded by an outer capsule.

The functions of the thymus were not well known until a few years ago. During autopsies it was noticed that young adults that had died in traumatic accidents often had much larger thymus glands than those dying from diseases of a chronic nature. The importance of the thymus in the immune system was discovered by Miller[287] who surgically removed the thymus from three day old mice and observed the subsequent deficiency in a lymphocyte population. There are several immune substances in the body and it is difficult to predict all the potential interactions when ingesting a thymus glandular. Furthermore, there are wide variations in response between different people and different animals.[288]

Thymus glands are available only from young animals (lambs and calves). The glands are covered by a capsule of fibrous connective tissue that penetrates the gland and divide it into lobules. The amount of connective tissue and fat increases with animal's age.

Thymus gland of neonatal calves is a source of many products. The thymus is responsible for the production of T-lymphocytes, as well as the production of various hormones including thymosin, thymopoeitin, thymulin, thymic humoral factor, and serum thymic factor.

Thymosin

Thymosin α 1 is believed to be a major component of Thymosin Fraction 5[289] responsible for restoring immune function in animals lacking thymus glands.[290] Thymosin alpha 1 has been used for a number of years in cancer treatment[291] in order to increase the body's immune system. It is now approved in 40 countries for the treatment of Hepatitis B and C.[292]

Currently, a variety of other active ingredients of the thymus gland are being tested in

287. Miller, J. F. (2004), "Events that led to the discovery of T-cell development and function: A personal recollection", *Tissue Antigens*, 63 (6): 509-17.

288. For more notices see: Pearse G. (2006), "Normal Structure, Function and Histology of the Thymus", *Toxicologic Pathology*, 34 (5): 504-514.

289. Thymosin fraction 5 contains several distinct hormonal-like factors which are effective in partially or fully inducing and maintaining immune function (See: Low T. L., G. B. Thurman, C. Chincarini, J. E. McClure, G. D. Marshall, S. K. Hu, A. L. Goldstein (1979), "Current status of thymosin research: evidence for the existence of a family of thymic factors that control T-cell maturation", *Annals of the New York Academy of Sciences*, 332: 33-48.

290. Garaci E. (2007), "Thymosin alpha 1: a historical overview", *Annals of the New York Academy of Sciences* ("Thymosins in Health and Disease First International Symposium", Pages xi–xii, 1–468), vol. 1112: 14–20; Li J., C. Hui Liu, F. Shan Wang (2010), "Thymosin alpha 1: Biological activities, applications and genetic engineering production", *Peptides*, 31 (11): 2151–2158.

291. Garaci E., F. Pica, G. Rasi, C. Favalli (2000), "Thymosin alpha 1 in the treatment of cancer: from basic research to clinical application", *International Journal of Immunopharmacology*, 22 (12): 1067–1076; Schulof R. S., M. J. Lloyd, P. A. Cleary, S. R. Palaszynski, D. A. Mai, J. W. Cox Jr, O. Alabaster, A. L. Goldstein (1985), "A randomized trial to evaluate the immunorestorative properties of synthetic thymosin-alpha 1 in patients with lung cancer", *Journal of Biological Response Modifiers*, 4 (2): 147-158.

292. Garaci E., C. Favalli, F. Pica, P. Sinibaldi Vallebona, A. T. Palamara, C. Matteucci, P. Pierimarchi, A. Serafino, A. Mastino, F. Bistoni, L. Romani, G. Rasi (2007), "Thymosin alpha 1: from bench to bedside", *Annals of the New York Academy of Sciences*, 1112: 225–34.

terms of their efficacy, such as thymosine beta 4, thmostimuline, and others. Thymus peptides are used for the treatment of immuno-deficiency diseases.

Thymus extract

Thymus extract[293] is used for infectious diseases including recurrent respiratory infections, colds, flu, H1N1 "swine" flu, hepatitis B, hepatitis C, Epstein-Barr virus (EBV), mononucleosis, herpes and shingles, sinusitis, and AIDS/HIV. It is also used for asthma, hay fever, food allergies, cancer, rheumatoid arthritis (RA), chronic fatigue syndrome (CFS), and systemic lupus erythematosus (SLE). Other uses include maintaining white cell production in cancer patients treated with radiation or chemotherapy and preventing the effects of aging. An increasing number of manufacturers offer thymus extracts for various diseases.

Thymus is also used in cosmetics to possibly slow skin aging processes and in hair care products because of its film-forming properties and peptides and amino acids.

For foods

The thymus glands from lamb and calf are blanched to firm the tissue and peeled from the capsule before they are cooked by frying or stewing.

When animal thymic tissue is sold in a butcher shop or at a meat counter, thymus is known as sweetbread.[294]

h) Thyroid

All vertebrates have a thyroid gland that is extremely vascular.[295] In mammals, it is usually bilobed and located just caudal to the larynx and adjacent to the lateral surface of the trachea. The two lobes may be connected by a fibrous isthmus (e. g., ruminants, horses, etc.), or a connecting isthmus may be indistinct (e.g. dogs, cats).[296] This gland produces, stores, and secretes the thyroid hormones that are the only iodinated organic compound in the body necessary for growth and proper metabolism.

Thyroid tissue is composed of millions of tiny saclike follicles, which store thyroid hormone, namely thyroglobulin, a glycoprotein. Blood capillaries attached to the gland yield a constant supply of plasma. Thyroglobulin is the chief component of the colloid, a jellylike substance that is secreted by the follicles. It attaches to the thyroid hormone for storage purposes. Before it is released into the bloodstream, the thyroid hormone is converted into

293. Thymus extract is a substance that is collected from the thymus gland of a cow, but it also can be manmade. The extract is believed to produce multiple health benefits, including boosting the human immune system. The use of thymus extract is known to posses certain risks. Since the extract is collected from the thymus gland of cows, there is a risk of contamination when the extract is collected from a diseased animal: the infection may transfer to humans.

294. "Sweetbread" is the name of a dish made of the animal's brain, pancreas, or thymus gland (neck/throat/gullet sweetbread) of an animal younger than one year old.

295. Among others see: Stathatos N. (2006), *Anatomy and Physiology of the Thyroid Gland*, in *Thyroid Cancer. A Comprehensive Guide to Clinical Management*, L. Wartofsky, D. Van Nostrand (Edts.), 2nd ed., 2006, Humana Press, Totowa, New Jersey (USA), pp. 3-7.

296. Reece W. O. (2009), *Functional Anatomy and Physiology of Domestic Animals*, 4° Edition, Wiley-Blackwell, Ames, Iowa (USA), pp. 165-7; Swindle M. M. (1998), "Comparative anatomy and physiology of the pig", *Scandinavian Journal of Laboratory Animal Science*, 25 (1): 11-21.

thyroxine[297] and small quantities of the other thyroid hormones. The quantity of thyroxine production depends on a sufficient intake of iodine and on stimulation by the thyroid-stimulating hormone (TSH) sent from the pituitary gland. Secretion of thyroid hormones, mostly thyroxine (T4), is controlled by thyroid-stimulating hormone (TSH), which is released by the pituitary gland when the level of thyroid hormones in the blood drops below a certain threshold.

In other words, the thyroid gland produces the hormone thyroxine, known as T4. The gland also secretes 3,5,3′-triiodothyronine (known as T3), and other deiodinated metabolites. T3 is about 3-5 times more potent than T4, while reverse T3 is thyromimetically inactive.

In conclusion, the body, when functioning properly, converts some of its thyroxine (T4) to triiodothyronine (T3), which is the major active thyroid hormone. Other hormones called T2 and T1 are also formed, although their precise functions are not yet fully understood. In the bodies, about 80% of triiodothyronine derive from conversion of thyroxine, a process mediated by deiodinase enzymes, the remainder coming directly from the thyroid gland.

Metabolic disorders happen when the thyroid secretes too little (hypothyroidism)[298] or too much thyroxine (hyperthyroidism). The first occurs when there is insufficient iodine in the diet. Excessive secretion of thyroxine known as myxedema in the adult and cretinism in infancy and childhood, comes from glandular malfunction.

The thyroid gland also produces the hormone calcitonin, a compound that stimulates deposition of calcium from the blood into the bones, balancing the action of parathyroid hormone.

Thyroid extracts

The treatment of hypothyroidism is to administer of thyroxine. Natural thyroid extracts have been used since 1891, when Murray[299] used an extract, made from dried animal glands to treating of myxedema. This use was approved in USA by the FDA in 1939.

Desiccated thyroid extract was the most common form of treatment for hypothyroidism until the discovery of the individual thyroid hormones (T3 and T4) and their commercial

297. Tyroxine is a precursor of thyroid hormone and the neurotransmitters dopamine, norepinephrine, and epinephrine. A deficiency of tyrosine leads to hypothyroidism and low adrenal function. The recommended daily amount of tyroxine is about 1 g/day for adults.

298. Some disorders related to thyroid deficiency are depression, poor concentration, memory disturbances, cold hands and feet, accumulation of excess body fat, dry skin, difficulty in losing weight, menstrual problems, thin hair, elevated cholesterol, migraine headaches, infertility and hypertension (See: Stanosz S. (1992), "Levels of thyroid hormones and thyrotropic hormone in serum of women with perimenopausal arterial hypertension", *Ginekologia Polska*, 63 (3): 130-3; Saito I., K. Ito, T. Saruta (1983), "Hypothyroidism as a cause of hypertension", *Hypertension*, 5: 112-115; Moreau T. (1988), "Headache in hypothyroidism. Prevalence and outcome under thyroid hormone therapy", *Cephalalgia*, 18: 687–9; Silva J. E. (2001), "The multiple contributions of thyroid hormone to heat production", *The Journal of Clinical Investigation*, 108 (1): 35–37; Fazio S., E. A. Palmieri, G. Lombardi, B. Biondi (2004), "Effects of Thyroid Hormone on the Cardiovascular System", *Endocrine Reviews*, 59 (1): 31-50; Vierhapper H. H. (1997), "Assessment of thyroid gland function in unwanted infertility--indications for TRH test and clinical impact from the viewpoint of the endocrinologist", *Acta Medica Austriaca*, 24 (4): 133-5; Heymann W. R. (2008), *Thyroid Disorders with Cutaneous Manifestations*, Springer-Verlag, London).

299. Murray G. R. (1891), "Note on the treatment of myxoedema by hypodermic injections of an extract of the thyroid gland of a sheep", *British Medical Journal*, 2: 796-7.

availability. During the 1960s, science-based physicians stopped using desiccated thyroid extract because its potency varied from batch to batch, which caused difficulty optimizing the patient's thyroid hormone levels. In the early 1960s, the development of synthetic thyroid hormones overshadowed animal hormones in doctor approval. Since then, animal thyroid hormones have taken a back seat to synthetic hormones. Today, desiccated thyroid[300] is made from pig thyroid glands.

Currently the opinions on the use of the two products (natural thyroid extracts and synthetic thyroid hormones i.e. thyroxine-T4) are different because they have both strengths and weaknesses.

The UK Royal College of Physicians has recommended the exclusive use of synthetic levothyroxine for the treatment of hypothyroidism. Synthetic pills contain a pure form of the hormones produced in the body, as it consist solely of T4. While they are called "synthetic", these products are actually identical to the natural compound T4.

At the present time, at least in Europe, a large majority of people of all ages who have hypothyroidism are being treated with levothyroxine.

.In animal-originated thyroid pills, the amounts of T4 and T3 vary among manufacturers. Moreover, the preparations from any single manufacturer over time will also vary. A perfect dose one month may be too little or too much a couple of months later, when the next batch comes on the market. It follows that desiccated thyroid extracts are not as precise as synthetic compounds. In addition, it is difficult to prescribe the ideal hormone dosage. Furthermore the hormone balances in animals are not the same as in humans, so it is not in the human's best interest to argue that animal thyroid hormones are truly natural. Many doubts surround the idea that tissue from an animal thyroid gland will migrate to the same organ site in the human body when ingested, and the idea assumes that digestion would not destroy the tissue. Animal thyroid pills aren't purified, which means that they contain substances that aren't naturally found in humans. Researchers have yet to determine how these substances affect the human body.

Finally, it should be remembered that in 2005, FDA enforcement removed more than half a million bottles of "Armour Thyroid" from US pharmacies due to unstable concentrations of thyroid hormone in the preparation.

Opposing opinion regarding the use of thyroid extract

The advocates of the use of animal thyroid extract have an opposing opinion. According to them thyroid extracts made from a healthy thyroid gland are better than synthetic thyroxine because they contain both T4 and T3 and unmeasured amounts of diiodothyronine (T2), monoiodothyronine (T1), calcitonin, and other protein-bound iodine.

Natural thyroid extracts containing T4, T3, and T1, T2, even if they derive from animals (pig, cattle, etc.), are identical in molecular structure to the human hormones. Therefore, there are many advantages in using them. Instead, synthetic preparations consist solely of T4 and depend on the body to convert the T4 to T3 and other metabolites. For those peo-

300. Desiccated thyroid is the dried and powdered thyroid gland. During the process of preparing this glandular, the fat and connective tissue are removed. Desiccated thyroid is often from hogs, but may also from cows and sheep. The pharmaceutical preparation is standardized and contains both thyroxine and triiodothyronine. "Armour Thyroid" or "Nature-Throid" & "Westhroid" the name of a commercial brand in USA.

ple whose problem is not a decline in T4 production but a defect in the conversion of T4 to T3, taking T4 may be useless.[301] Moreover, the various thyroid hormones surely exist in the body for a reason, so supplementing with the thyroid hormone spectrum seems necessary.

Harrower[302] was one of the leading advocates of the "Organotherapy" approach.[303] According to him, "clinical experience has established this beyond all doubt - literally thousands of cases of dyscrinism[304] having been treated with single endocrine products without satisfactory results have, on changing to an indicated pluriglandular remedy, shown results so different that they are remarkable."

It should be noted that even the effect of soy on thyroid function is currently a controversial topic. Some believe that soy increases metabolic rate and thyroid function, others say it has no effect.[305] Several recent articles, however, have noted problems with people taking both soy and thyroid supplements at the same time.[306]

Divi and others[307] have identified the mechanism of soy's effect on thyroid function. Genistein and daidzein, the isoflavones in soy, inhibited thyroid peroxidase by acting as alternative substrates. They conclude that "because inhibition of thyroid hormone synthesis can induce goiter and thyroid neoplasia in rodents, delineation of anti-thyroid mechanisms for soy isoflavones may be important for extrapolating goitrogenic hazards identified in chronic rodent bioassays to humans consuming soy products."

4.7 Hides and skins

The largest components, based on value and volume, of ABPs derived from the slaughter of food animals are the hides and the skins,[308] in particular those derived from cattle. Animal hides have been used for shelters, clothing and as containers (water, foods, etc.) since prehistoric times. Hides and skins are generally one of the most valuable by-products from animals.

301. Bunevičius R., G. Kažanavičius, R. Žalinkevičius, A. J. Prange Jr. (1999), "Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism", *New England Journal of Medicine*, 340 (6): 424-9.

302. Harrower H. R. (1922), *Practical organotherapy: the internal secretions in general practice*, The Harrower Laboratory, Glendale CA, p. 34.

303. Harrower H. R. (1914), Practical Hormone Therapy: A Manual of Organotherapy for General Practitioners, Hueber Verlag GmbH & Co K, Ismaning (D).

304. It is a term used for any endocrine malfunction.

305. Dillingham B. L., B. L. McVeigh, J.W. Lampe, A.M. Duncan (2007), "Soy protein isolates of varied isoflavone content do not influence serum thyroid hormones in healthy young men", *Thyroid*, 17 (2): 131-137.

306. Messina M., G. Redmond (2006), "Effects of Soy Protein and Soybean Isoflavones on Thyroid Function in Healthy Adults and Hypothyroid Patients: A Review of the Relevant Literature", *Thyroid*, 16 (3): 249-258; Setchell K. D. (1998), "Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones", *American Journal of Clinical Nutrition*, 68: 1333S-46S; Conrad S. C., H. Chiu, B. L. Silverman (2004), "Soy formula complicates management of congenital hypothyroidism", *Archives of Disease in Childhood*, 89 (1): 37-40.

307. Divi R. L., H. C. Chang, D. R. Doerge (1997), "Anti-Thyroid Isoflavones from Soybean: Isolation, Characterization, and Mechanisms of Action", *Biochemical Pharmacology*, 54 (10): 1087–1096.

308. Wordreference is implying that both Hide and Skin are synonyms, having roughly the same meaning. But in the current language "hides" and "skins" are two different parts of the same animal: the difference is that hides are thicker: we would say cowhide and pigskin.

The hides represent a great portion of the weight of the live animal: from 4% to as much as 11%.[309] After the hide or skin is removed from the animal, it should be cured[310] quickly to avoid decomposition by bacteria and enzymes.

There are four basic treatments: airdrying, curing with salt, curing by mixer and curing by raceway.[311] Some skins, such as sheepskins and pigskins, contain a large quantity of fat. It is desirable to reduce the fat to approximately 3.0% on a dry-weight basis. It takes four weeks to process raw hides into leather.

The skin of virtually every animal can be used to produce leather. Animal skins have been the source of clothing attire for man since prehistoric times. Leather is used in a remarkable number of applications, including automobile and furniture upholstery, shoes, sporting goods, luggage, garments, gloves, and purses. A representative of the leather industry categorized leather utilization as 40% for upholstery, 50% for shoes and shoe leather, and 10% for other uses.

Slaughterhouse skin by-products can be treated and become a good protein source. The skin is shredded into little pieces and washed. The wash water is filtrated for impurities and the dissolved proteins are concentrated. These concentrated proteins are mixed back into the clean and centrifuged skin-shreds; the skin-shreds absorb the concentrated protein and can be dried. The dry material will contain approx. 7% of water and have an acceptable industrial shelf life.

The skin of some animal species is also used for processed meat products. This is the case with pork skin and poultry skin, in some cases also with calf skin, particularly from calf heads and legs.[312]

Gelatin

Animal gelatin[313] is an edible jelly produced by the controlled hydrolysis of a water-insoluble collagen derived from proteins extracted from fresh animal tissues (mainly skins and bones) that have arrived in an edible condition, through boiling. Both hides and bones contain large quantities of collagen. Commercially available gelatin is a dry powder of various granule sizes. Mixed with water the protein molecules of the gelatin absorb water and form a gel.

The processing of gelatin from hide consists of three major steps: elimination of non collagenous material from the raw material, controlled hydrolysis of collagen to gelatin, recovery and drying of the final product.

309. E.g. cattle: 5.1 - 8.5%, average: 7.0%; sheep: 11.0 - 11.7%; swine: 3.0 - 8.0%.

310. Long ago, the Native Americans used wood, woodsmoke and animal brain tissue as tanning agents to cure and preserve animal hides.

311. Anderson R. M. (1948), *Methods of Collecting and Preserving Vertebrate Animals*, National Museum of Canada, Bulletin No. 69, Ottawa; O'Flaherty F., W. T. Roddy, R. Miller Lollar (1978), *The chemistry and technology of leather*, R. E. Krieger Pub. Co., Huntington, N.Y; Thorstensen T. C. (1985), *Practical leather technology*, R. E. Krieger Pub. Co., Huntington, N.Y; Minnoch J. K., S. R. Minnoch (1979), *Hides and skins*, National Hide Association, Chicago 6, Ill-USA.

312. *Chicharrón* (Spanish, Portuguese: *Torresmo*, Filipino: *Chicharon*) is a food made of fried pork rinds. It is sometimes made from chicken, mutton, or beef.

313. Lambert T. (2008), Glue, Gelatine and Their Allied Products: A Practical Handbook for the Manufacturer & Agriculturist, BiblioBazaar, Charleston (USA); The Manufacture of Glue and Gelatine; the Application and Uses of Machinery, Etc. New York, Chicago, etc., National Provisioner Publishing Co. General Books, 2010.

Animal gelatin is either spray dried as simple gelatin or as a hydrolyzed gelatin, i.e. partial or total splitting of the long gelatin molecules into smaller fragments. This is done with chemical or enzymatic processes followed by refining, and other unit operations, to obtain the required product cleanness and or property. To ensure an economically feasible drying process, all gelatin products are concentrated before drying. After being concentrated, the gelatin is dried by one of many methods, such as being cooled in a drying tunnel, drum drying or spray drying.

Another extraction procedure for gelatin is acid processing. This is usually applied to pig skin or bone.

Gelatins are used in the food and pharmaceutical industries. When it is used in food industries is added to a wide range of foods, forming a major ingredient in jellies and aspic. Its main use is the production of jellied desserts, because of its "melt in the mouth" properties, and it is is also added to a range of meat products, i. e. to meat pies. Gelatins are also widely used as a stabilizer for ice cream and other frozen desserts. They are added as a protective colloid to ice cream, yoghurt and cream pies. The raw material can also be rendered into lard. Collagen from hides and skins has a role as an emulsifier in meat products because it can bind large quantities of fat. This makes gelatins a useful additive or filler for meat products.

In USA and in Europe the pharmaceutical industry uses about 6.5% of the total production of gelatin. Most of it is used to make the outer covering of capsules and as a binding and compounding agent in the manufacture of medicated tablets and pastilles. It is used as ingredient in protective ointment, such as zinc gelatin for the treatment of ulcerated varicose veins.

Since gelatin is a protein, it is used as a plasma expander for blood in cases of very severe shock and injury. A product made from extracted collagen can stimulate blood clotting during surgery. Pork skin is similar to human skin, and can be converted into a dressing for burns or skin-ulcers. Prior to use, pork skin used as a dressing needs to be cut into strips or into a patch, shaved of hair, split to a thickness of 0.2 -0.5 mm, cleansed, sanitized and packaged.

Gelatin is an excellent emulsifier and stabilizing agent for many emulsions and foams. It is used in cosmetic products, in silk screen printing inks, and photogravure printing, etc.[314]

Pork skin

It is normally used as food, unlike other animal skins that are used in leather production. Nonetheless, pork skin is a sometimes used as a fabric for footwear and garments. Because it is similar to human skin, it can be converted into a dressing for burns or skin-ulcers.

Pork skin is collagen rich and in precooked form a valuable material for the manufacture of some meat products of the precooked-cooked variety. Occasionally raw pork skin is shredded into small particles and used in processed meat products . It can also be used for gelatin production.

Collagen, that is a fibrous protein abundant in all animals, is used in the food industry as casing and wraps. A wrapping film for hams and other food products can be made using collagen membranes from pig skin.

Skin glue manufacturing uses the parings and cuttings of hides from tan-yards: for example the ears of oxen and sheep, and the skins of rabbits, hares, cats, dogs and other animals. Supposedly the best skin glue is obtained from a mixture of the hide, ear and face clippings of the ox and calf. For art applications, rabbit, sheep and deer skin glue are popular.

314. GMIA, Gelatin Manufacturers Institute of America, *Gelatin Handbook*, Written and produced by the members of the GMIA, 2012.

Rabbit skin

Rabbit skin glue is made from rabbit skin, tendon, bone and gut and is used in making and repairing instruments, traditional woodworking, gilding and painting techniques.

Chicken skin

Chicken skin is removed from the carcass or from individual cuts. It has a high fat content and is ground prior to being added to processed meat products. Chicken fat serves as the fat portion in all-chicken processed meat products (chicken frankfurters, chicken nuggets, and chicken bologna). Chicken skin is added to meat products for the same purpose as pork fat in pork/beef products, namely to contribute to product flavor and produce a soft texture.

4.8 Hairs and wools, nails, horns, feathers, hooves

Keratin

Keratin is one of the main components of these products. The earliest documented use of keratin in medicine comes from a Chinese herbalist named Li Shi-Zhen in the Ming Dynasty, as recalled by Ben Cao Gang Mu.[315] Around 1849, the word "keratin" appears in the literature to describe the material that made up hard tissues such as animal horns and hooves. In the following years many scientists were more interested in dissolving hair and horns in order to make better products. In the years preceding World War I, lime was applied to the manufacture of keratin gels.[316] From 1900 to 1940, several methods were developed to obtain keratins using oxidative and reductive chemistries.[317] Among the first inventions were keratin powders for cosmetics, composites, and coatings for drugs, respectively.[318] It was during the years of World War II and after that one of the most comprehensive research projects on the structure and chemistry of hair fibers was undertaken: between the years of 1940 and 1970, nowhere in the world was keratin research more active than in Japan. During the same years in Australia, the Council for Scientific and Industrial Research (later the Commonwealth Scientific and Industrial Research Organisation or CSIRO) was founded in order to better understand the structure and chemistry of wool fibers. Researchers at CSIRO conducted many of the most fundamental studies on the structure and composition

315. Ben Cao Gang Mu (本草綱目), *Materia Medica, a dictionary of Chinese herbs*, written by Li Shi Zhen (李時珍) (1518-1593). It consists of 52 volumes. The book lists 1,892 medical material of herbs, animals and mineral with 11,096 formulae being used in the past. The book has been translated into more than 60 languages (Japanese, French, German, Russian, Latin, etc..).

316. In a German patent issued in 1905 (n. 184,915), John Hoffmeier described a process for extracting keratins from animal horns using lime.

317. Brein F., O. Baudisch (1907), "The oxidative breaking up of keratin through treatment with hydrogen peroxide", *Zeitschrift für Physiologische Chemie*, 52: 158-69; Neuberg C., *Process of producing digestible substances from keratin*, US pat. n. 926,999, 1909; Goddard D. R., L. Michaelis (1934), "A study on keratin", *The Journal of Biological Chemistry*, 106: 605-14.

318. Beyer C., *The keratin or horny substance of the hair*, German pat. N. 22,643, October 14, 1907; Goldsmith B. B., *Thermoplastic composition containing keratin*, US pat. N. 922692, May 25,1909; Dale H. N. (1932), "Keratin and other coatings for pills", *Pharmaceutical Journal*, 129: 494-5.

of wool. In 1965, CSIRO scientist Crewther and others[319] published the definitive text on the chemistry of keratins.

During the 1980's, a number of scientists began to explore the potential uses of keratins. In 1982, Japanese scientists[320] published the first study describing the use of a keratin coating on vascular grafts as a way to eliminate blood clotting, as well as experiments on the biocompatibility of keratins.[321] Soon thereafter in 1985, two researchers from the UK published a review article speculating on the prospect of using keratin as the building block for new biomaterials development.[322] A review by Rouse and Van Dyke[323] stated that extracted keratins were capable of forming self-assembled structures that regulated cellular recognition and behavior. The studies and discovered qualities led to the development of keratin biomaterials with applications in wound healing, drug delivery, tissue engineering, trauma treatment and medical devices manufacturing.

In addition, these and others studies concluded that keratins were a group of strong fibrous, structural proteins and were the key structural material making up the outer layer of human and animal skin. Keratins are also the key structural component of hair and nails. Keratin amino acids have several unique properties and, depending on the levels of the various amino acids, they can be inflexible and hard like hooves, or soft as is the case with skin. The polypeptide chains of keratin are arranged in parallel sheets held together by hydrogen bonding. Approximately 10 keratins form the basis of hair or claw, with a further 20 found in internal body cavity epithelia.[324] In humans 54 functional keratin genes exist.[325] Cysteine is a major component of keratin proteins; it is an attractive material for the absorption of heavy metals from aqueous solution.

Keratin compounds can be hydrolyzed to form many useful materials and numerous patents, especially in USA and Japan.[326] have been issued.

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319. Crewther W. G., R. D. B. Fraser, F. G. Lennox, H. Lindley (1965), *The Chemistry of Keratins*, in C. B. Anfinsen Jr., M. L. Anson, J. T. Edsall, and F. M. Richards (Edts), *Advances in protein chemistry*, Academic Press, New York, pp. 191-346.

320. Noishiki Y., H. Ito, T. Miyamoto, H. Inagaki (1982), "Application of denatured wool keratin derivatives to an antithrombogenic biomaterial: Vascular graft coated with a heparinised keratin derivative", *Kobunshi Ronbunshu*, 39 (4): 221-7.

321. Ito H., T. Miyamoto, H. Inagaki, Y. Noishiki (1982), "Biocompatibility of denatured keratins from wool", *Kobunshi Ronbunshu*, 39 (4): 249-56.

322. Jarman T., J. Light (1985), "Prospects for novel biomaterials development", *World Biotechnology Rep*, vol. 1, pp. 505-12.

323. Rouse J. G., M. E. Van Dyke (2010), "A Review of Keratin-Based Biomaterials for Biomedical Applications", *Materials*, 3: 999-1014.

324. The first to point out that there are several types of keratins was Lissizin T. (1928) ("The oxidation products of keratin by oxidation with permanganate II", *Zeitschrift für Physiologische Chemie*, 173: 309-11).

325. Moll R., M. Divo, L. Langbein (2008), "The human keratins: biology and pathology", *Histochemistry and Cell Biology*, 129 (6): 705–733.

326. Among the most interesting: US Patents n. 3,642,498 (1972); 4,240,450 (1980); 4570629 (1986); 5047249 (1991); 6,270,791 (2001); 6,316,598 (2001); 6,270,793 (2001); 6,274,155 (2001); 6.461,628 (2001). Japan Patents Appl. n. S62-333838 (1989); 3-223207 (1991) 04082561 (1992); 247925 (2008); 023924 (2009).

Feathers

Internationally, feathers production is huge.[327] Feathers are used in a number of decorative products, such as boas, feather fans, masks, costume accessories, bird ornaments and even earrings and flowers. Down and feathers are used for insulation and padding of products like jackets, bedding, sleeping bags, and pillows. Most of these products contain a blend of down and feathers. The production and harvesting of fine feathers and down for use in the garment and household linen industries is different than the processing of coarse feathers for feather meal. In the USA much of the production of coarse product is converted into feather meal that is hydrolyzed for use in animal feed supplements, fertilizers and other products. Since post World War II, hundreds of patents have been issued for these uses (insulating products, fiber products, oil coagulants, pest repellent, etc.). Dalev[328] developed and promoted a combined enzyme-alkaline technology for processing waste feathers from poultry slaughterhouses. On the other hand, Kumar and others[329] have experimented with the use of a type of bacillus to biodegrade poultry feathers.

Horns and hooves

Horns and hooves are used as raw material for fertilizers that are excellent source of slow release nitrogen; for the production of protein based fire-fighting agents; for making foaming agents; and for light weight cellular concrete additives.

Wool and hair

Wool and hair have multiple uses based on their fiber properties. These qualities guide their usage into fabric, building insulation, and absorptive products. Synthetically derived products have challenged hide, skins, wool, and hair in nearly all traditional applications and will undoubtedly continue to do so in the future.

Fire fighting foams

Hooves, horns, and feathers can be useful to make fire fighting foams. Fire fighting foams are a collection of bubbles formed by the aeration of a foam concentrate solution with water. It follows that foams are made up of three components: foam concentrate (a liquid

327. World-wide poultry processing plants are producing millions of tons of feathers as waste products annually. Feathers represent 5-7% of the total weight of mature chickens. Around 24 billion chickens are being killed per year across the world which is discarding about 2 million tonnes of poultry feather. Feather is generated in bulk quantities as a by-product of poultry industry. Typically as each bird has up to 125gms of feather, the year worldwide production of feather waste is about 155.000 tons. As regards the geese, they moult the natal plumage into juvenile feathers between 3-5 weeks of age and than moult that juvenile plumage into adult plumage between 8-11 weeks of age. Feather weight of an adult goose makes up about 6.2% of its total body weight (See: Kozák J. (2011), "An Overview of Feathers Formation, Moults and Down Production in Geese", *Asian-Australasian Journal of Animal Sciences*, 24 (6): 881–887).

328. Dalev P. G. (1994), "Utilisation of waste feathers from poultry slaughter for production of a protein concentrate", *Bioresource Technology*, 48 (3): 265–267.

329. Kumar E. Vijay, M. Srijana, K. Chaitanya, Y. H. Kumar Reddy, G. Reddy (2011), "Biodegradation of poultry feathers by a novel bacterial isolate Bacillus altitudinis GVC11", *Indian Journal of Biotechnology*, 10: 502-507.

produced by chemical manufacturers and supplied in drums or bulk), water and air. Generally, the task of fire fighting is to cool the fire and to coat the fuel, preventing its contact with oxygen, in order to suppress the combustion.[330] Fire-fighting foam, floats on a fuel surface separating it from oxygen. Other components of fire-retardant foams are organic solvents, foam stabilizers, and corrosion inhibitors.

Over the years, foams have been classified in different ways. The earliest foams were based upon a chemical reaction occurring between aluminum sulphate and sodium bicarbonate. This type of foam is now largely obsolete. Nowadays there are two types of foams: synthetic and protein foams.

The first type is made, commonly, from a mixture of diammonium sulphate, diammonium phosphate, monoammonium phosphate, besides gum thickeners, an iron oxide coloring agent, and preservatives:[331] for long-term fire retardants fertilizer salts, are typically mixed in with water to ensure uniform dispersal. Even after the water has evaporated from these synthetic foams, the retardant remains effective until it is removed by rain or erosion.[332] The foams form a combustion barrier after the evaporation of the water carrier, and its effectiveness depends on the amount of retardant per unit surface area. There are other types of synthetic foam forms based on mixtures of non-fluorochemical, hydrocarbon type surfactants along with solvents and water. These foam agents do not form aqueous films or polymeric membranes. Instead, they function by forming an aggregate of foam bubbles on the surface of the fuel.

Protein foams were the first types of mechanical foam to be marketed extensively and have been used since World War II.[333] These foams are produced by the hydrolysis of granulized keratin[334] protein (protein hydrolysate) such as hoof and horn meal, chicken feathers, etc. They produce a homogeneous, stable foam blanket that has excellent heat resistance, burnback, and drainage characteristics. All contain surfactants, foaming, and wetting agents. Stabilizing additives and inhibitors are included to prevent corrosion, resist bacterial decomposition and to control viscosity. Fire retardants depend on the water they contain to retard or suppress the fire. The foaming agents affect the rate at which water drains from the foam and how well it adheres to the fuel. The surfactants and wetting agents increase the ability of the drained water to penetrate fuels thus reducing their ability to ignite. Fuels are insulated from heat and air contact is reduced. These retardants lose their effectiveness once the water has evaporated or drained from them.

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330. NF-National Foam, *A Firefighter's Guide to Foam* (www.Kidde-Fire.com); "Ideas Advance Fire Fighting", *Industrial Fire World Magazine*, 2005, 20 (6): 10-12, 36-37.

331. Hamilton S., D. Larson, S. Finger, B. Poulton, N. Vyas, E. Hill (1998), *Ecological effects of fire retardant chemicals and fire suppressant foams*, Northern Prairie Wildlife Research Center Home Page, Jamestown, (ND-USA).

332. U.S. Department of Agriculture, Fire fighting chemical products - Differences and similarities, Wildland Fire Chemical Systems, Missoula Technology and Development Center. U.S. Forest Service. Missoula, (MT-USA) 2002. (http://www.fs.fed.us/rm/fire/Wildland_chemicals.html).

333. On the use of hooves and horns, to produce foams, have been issued numerous patents. One of the newest is the Patent Family Members CN 101491723 A, Protein foam fire extinguisher production method using hoof and horn grain (29-Jul-2009). The product of this invention seems to have the advantages of long storage period, high foam expansion rate, less sediment, long drainage time and high extinguishing efficiency.

334. The keratin helps to bond the foam bubbles into a durable blanket, which stops it breaking up on impact with the fire and makes it very effective at smothering flames.

Unlike synthetic foams, protein foams are bio-degradable, flow and spread slower, and provide a foam blanket that is more heat-resistant and more durable. They have slow knock-down characteristics but provide superior post fire security at very economical cost. They may be used with fresh or seawater. The foam is specifically designed to quell the hotter, high intensity fires triggered by aviation fuel and is therefore used to make a special fire extinguishing foam used by airport fire and rescue teams. Protein foams are especially intended for use on hydrocarbon fuels.

There is significant potential for damage to terrestrial vegetation and to aquatic ecosystems from synthetic fire fighting foams. The environmental effects of fire retardants have been of concern since the 1970s.[335] However, few studies into the ecological effects of fire retardants or foams on vegetation have been carried out.[336] Because the protein foams are biodegradable, the damage on the environment should be more limited.

4.9 Hearts

Heart is used as a table meat: it can be roasted, braised, grilled. Heart meat is often also used as an ingredient in processed meats. It can be also used for transplantation.

Xenotransplantation (XT) is the transplant of organs, cells, or tissues between species. It commonly refers to transplants of animal organs (e.g., nonhuman primates or pigs) into humans.[337]

Xenotransplantation is not a recent phenomenon.[338] Researchers have made sporadic attempts at cross-species transplants from early as the 17th century with little success. Primate donors were used in early whole organ experiments. Pig heart valves have been used in humans for several decades; however they are first chemically treated to kill pig cells.[339] In 1999, the U.S. FDA[340] banned clinical trials with non-human primates. On April 6th,

335. Dodge M. (1970), "Nitrate poisoning, fire retardants, and fertilizers-any connection?", *Journal of Range Management*, 23 (2): 44-247.

336. Between these: Gaikowski M. P., S. J. Hamilton, K. J. Buhl, S. F. McDonald, C. H. Summers (1996), "Acute toxicity of three fire-retardant and two fire-suppressant foam formulations to the early life stages of rainbow trout (Oncorhynchus mykiss)", *Environmental Toxicology and Chemistry*, 15 (8): 1365–1374; McDonald S. F., S. J. Hamilton, K. J. Buhl (1996), "Acute toxicity of fire control chemicals to Daphnia magna (Straus) and Selanastrum capricornutum (Printz)", *Ecotoxicology and Environmental Safety*, 33: 62-72; McDonald S. F., S. J. Hamilton, K. J. Buhl (1997), "Acute toxicity of fire-retardant and foamsuppressant chemicals to Hyalella azteca (Saussure)", *Environmental Toxicology and Chemistry*, 16 (7): 1370-1376; Ecological Risk Assessment: Wildland Fire-Fighting Chemicals, Prepared by LABAT Environmental for Missoula Technology and Development Center, USDA Forest Service, Missoula, (MT-UA) 2007.

337. Bühler L. H. (Edtr.) (2013), *Xenotransplantation*, John Wiley & Sons A/S, London.

338. Küss R., P. Bourget (1992), *Une histoire illustrée de la greffe d'organes. La grande aventure du siècle*, Laboratoires Sandoz, Rueil-Malmaison (France); Deschamps J.-Yves, F. A. Roux, P. Saï, E. Gouin (2005), "History of xenotransplantation", *Xenotransplantation*, 12 (2): 91–109; Barber N. (2007), *Organ Transplanting. The Cannibalistic Nature of Transplant Medicine*, Third Edition, Adelaide, Australia, (standardoil@hotmail.com).

339. For a detailed chart of all xeno experiments using cells or organs from non-human primates see the Council of Europe's July 2000 report on xenotransplantation (Tallacchini M., *Existing regulations on xenotransplants in the European area* (COE, EU and EEA countries), XENOME WP5, D274).

340. Butler D. (1999), "FDA warns on primate xenotransplants", *Nature*, 398 (6728): 549-637.

1999, the U.S. FDA stopped short of banning nonhuman primate xenografts, encouraging "further scientific research" into the risks. The European legal aspects are discussed by Taupitz and Weschka.[341]

Since the mid-1990s the European Institutions and the European Agency for the Evaluation of Medicinal Products (EMEA) adopted diverse xenotransplantation policies. In 1997, the Council of Europe (COE) approved Recommendation 15/97 on the necessity to set standards for xenotransplantation clinical trials. At that time, the European countries – those belonging to the Council of Europe, to the European Union (still European Communities at that time), and to the European Economic Area (EEA) – began taking steps to regulate XT.

In 1999, the Parliamentary Assembly of COE asked the Member States to take into account a moratorium on all clinical trials with XT, and some countries (such as Denmark, The Netherlands, and Norway) agreed; others set up dedicated institutions (UK) or technology assessment procedures (Switzerland) to improve control or understanding of the implications of XT.

In 2007, the approval of Regulation 2007/1394/EC has provided a direct regulation for xeno-cell and tissue-based products, having established a central approval procedure for the market placing of so-called advanced therapies.

From 1997 until 2009, the EU xenotransplantation policy was not subject to as fierce a public dispute and scrutiny at the European level as other areas of biotechnology and biomedicine. The European Council briefly discussed xenotransplantation in the context of the clinical trials Directive. On 25 September 2007, the European Parliament adopted a declaration urging the Commission and the Council of Ministers to end the use of apes and wild-caught monkeys and to establish a timeline to replace all non-human primates in scientific experiments.[342] The Scientific Committee on Health and Environmental Risks (SCHER) concluded that from a scientific point of view "developing and testing xenotransplantation methodologies" was one of four areas in which "the use of non-human primates (NHPs), at the present time, is essential for scientific progress". On 8 September 2010, the European Parliament adopted a new Directive, in agreement with the Council, to revise Directive 86/609/EEC on the protection of animals used for scientific purposes (Directive 2010/63/EU). It acknowledged that animals, including NHP, were still needed for scientific research.

On the other hand, the demand for human organs and tissues for transplantation far exceeded the world supply. For every person who benefits from an organ transplant, there are an estimated 5 to 10 other potential recipients for whom human organs are not available.[343] Xenotransplantation is viewed as a potential solution to the existing shortage of human organs for transplantation.

Furthermore, with limited success in terms of patient survival or organ functionality, experiments have been performed on transplants of bone marrow, hearts, neurons, cells[344]

341. Taupitz J., M. Weschka (Edtrs) (2009), Chimbrids chimeras and hybrids in comparative European and International Research. Scientific, Ethical, Philosophical and Legal Aspects, Springer-Verlag Berlin Heidelberg, pp. 98-100.

342. European Parliament (2007a): Declaration 0040/2007 on primates in scientific experiments. EP reference number: DCL-oo40/2007/P6_TA-PROV(2007)00407, (http://ec.europa.eu/environment/chemicals/lab_animals/ pdf/declaration_nhp_en. pdf).

343. Boneva R. S., T. M. Folks (2001), L. E. Chapman, "Infectious Disease Issues in Xenotransplantation", *Clinical Microbiology Reviews*, 14 (1): 1–14.

344. Sanal M. G. (2011), "Future of liver transplantation: non-human primates for patient-specific

and other tissues from baboons, chimpanzees and pigs. However, pig and cow heart valves have been commonly used in human patients.[345] Some heart patients owe their lives to valves from pig hearts.

Pigs are a promising donor species because their organs are similar in size to humans, they have large litters, grow quickly, and are far enough from humans on the evolutionary tree to minimize infection risks. They are socially since many in our society regularly consume them for food.

Despite several potential advantages over allotransplantation, xenotransplantation encompasses a number of problems. It is a common misconception that successful xenotransplants involve animals that are most like humans, i.e. chimpanzees. In fact, chimps are not so much like humans; and they are endangered, expensive to raise, and grow too slowly to be of use for the thousands needing transplants. For example, at Loma Linda University Medical Center in California, on October 26, 1984, a 2-week-old infant known as "Baby Fae" received a baboon heart transplant. She died after 20 days.

The potential to introduce infections across species barriers is another concern. Chimps are also so unlike humans that the risks of transplanting an infectious virus are too high.[346]

The human immune system reacts violently to pig organs and pigs contain ubiquitous retroviruses that may adversely affect humans. Many biotech companies are working to resolve these and other ethics issues that contribute to the xenotransplantation debate.

4.10 Intestines

The processing of animal intestines includes the following principal operations: separation of the intestinal complex into parts; removal of the intestinal contents and thorough washing of the intestines ; fat extraction (skimming) ; and removal of the mucous membrane (stripping) and other superfluous layers. The processed intestines are preserved with salt or by curing. Slaughterhouses that do not process animal intestines preserve incompletely processed (not cleaned and washed) intestines by corning (salting or soaking in brine). Salted intestinal finished products are packed in wooden barrels, and dried intestinal finished products are packed in wooden boxes. Intestines may be utilized for: sausage casing, strings (for suturing material, surgical ligatures, musical instruments, and racquets), and for use in the manufacturing of heparin, and of intestinal mucus (SIS materials).-

Sausage casings

Animal casings can be used for a variety of different purposes, such as surgical sutures, collagen sheets (used for burn dressing), strings for musical instruments, casings, human food, pet food, meat meal, tallow, and fertilizer.

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organs from induced pluripotent stem cells", *World Journal of Gastroenterology*, 17 (32): 3684-90.

345. Ekser B., P. Rigotti, B. Gridelli, D. K. Cooper (2009), "Xenotransplantation of solid organs in the pig-to-primate model", *Transplant Immunology*, 21 (2): 87-92.

346. Yoo D., A. Giulivi (2000), "Xenotransplantation and the potential risk of xenogeneic transmission of porcine viruses", *Canadian Journal of Veterinary Research*, 64 (4): 193-203; Boneva R. S., T. M. Folks, L. E. Chapman (2001), Ibid., 14 (1): 1-14; Aynaud J. M. (2000), "Risks of infection in xenotransplantation: what are they and how are they to be controlled?", *Pathologie Biologie* (Paris), 48 (4): 387-8; Meng X. J. (2003), "Swine hepatitis E virus: cross-species infection and risk in xenotransplantation", *Current Topics in Microbiology and Immunology*, 278: 185-216.

Certainly the area having the highest economic value combined with volume of utilization would be sausage casings. For these purposes sausage casings must be processed in a specific way.[347] Some of them are used as container for the sausage mix and peeled off before consumption while others are eaten with the sausage.

Both natural and artificial casings are soft cylindrical tubes used to contain sausage mixes. Natural casings are obtained from animal intestines[348] derived from slaughtering. Areas of the animal used to manufacture casings are the small and large intestine, gullet and throat, urinary bladder, stomach, and rectum from most meat-producing animals. Many factors influence the quality of the casing, such as health of the animal, specie, age of the animal, fodder consumed, breed, conditions under which the animal was raised, portion of the intestinal tract utilized, and how the product is handled and processed after the animal is slaughtered. Casings are mainly derived from the small and large intestines of sheep, goats and pigs, but also from cattle and horses. Small caliber natural casings are derived from the small intestines of sheep, goats and pigs. They are processed in a way that makes them tender and edible and are usually eaten with the sausage. The small intestines are detached from adhering mesenteric tissue. The intestinal content is removed manually and the empty casings is flushed with water and subsequently de-slimed by using either manual or electrically operated casing-cleaning machines.[349]

Many other parts of the intestinal tract of slaughter animals can also be used for natural casings but they are processed differently due to stronger and tougher casing walls. Because of the hardness and toughness, those parties are generally considered unfit for human consumption and are usually peeled off before consuming the sausages.

Only the small intestines of the gastro-intestinal tract of sheep and goats are normally processed to be used as casings for many types of products (e. g. fresh frying sausages, frankfurters, BBQ sausages, hot dogs and thin dried fermented sausages). These casings are processed in a way that they become tender enough to be easily chewed.

Regarding pig intestines, several parts are processed into casings, and the most important is the small intestines. The large intestines having an average length of 3 m (pig middles) and the cap are used as casings for coarse liver sausage and sometimes for salamis. The "bung", the last part of the gastro-intestinal (average length of 0.8 m), is notable for its strength and shape and is used as casing for products such as cervelat[350] and fine emulsified liver paste. The pig bladder can also be used for products such as black pudding or gelatinous meat mixes.

347. Among others see: Gerrard F. (1969), *Small goods production: a practical handbook on the manufacture of sausages and other meat products*, Leonard Hill Books, London; Gayler P. (2011), *Sausages*, Jacqui Small, London.

348. Anatomically the walls of the intestinal tract of slaughter animals consist of four layers of intestinal tissue. These layers from inside to outside are: Mucose membrane (I), submucose membrane (II), muscular layer (circular and longitudinal) (III) and serose membrane (IV). For natural casing manufacture, one or more of these layers are removed during casing processing depending on the type of casing (thin/thick, edible/non-edible) to be fabricated.

349. The small intestines are passed through a set of rollers to loosen the tissue layers and to remove the "slime" that is the internal layer of the intestine, basically the internal mucose or membrane. In the slaughtered animal this membrane disintegrates rapidly and can easily be removed.

350. Cervelat, also cervelas, servelat or zervelat is a kind of finely chopped dry fermented sausage produced mainly in Switzerland, Alsace and in parts of Germany.

As regards cattle, many parts of their gastro-intestinal tract are used as casings in sausage production: "rounds" (small intestines)[351] are used for stuffing sausages such as lyoner[352], liver and blood sausages and dried fermented beef products: the "middles"[353] are used for dried fermented and precooked-cooked sausages such as "hunter's sausage" and coarse liver sausage; the "blind gut" is also used for precooked-cooked sausages and raw-cooked products such as large bologna, etc. Beef bladders are used for "mortadella" and other specialties. These natural casings are usually not eaten owing to their tough casing walls, although they are edible.

Artificial casings are made of cellulose, collagen, and synthetic materials. They were developed in response to the growing meat industries at the beginning of the 20th century. At that time, in some countries, the supply of natural casings could no longer cope with the demand for such natural casings. Artificial casings showed several advantages compared to natural casing, as for example the negligible microbial contamination unnecessary refrigeration, and absence of spoilage problems during transport and storage. Nowadays, for wide sausage calibers, artificial casings are the material of choice, while for smaller caliber products, artificial and natural casings remain equally important.

According to their structure and composition of material, artificial casings can be subdivided into

1) casings made of natural materials, with two groups:
   a) casings made of organic plant material, namely cellulose;
   b) casings made of animal by-products, namely collagen.
2) casings made of synthetic substances deriving from thermoplastic materials which can be subdivided into "polymer casings" and "plastic casings".

Collagen casings

This type of casings is fabricated from collagen,[354] which is obtained from the corium layer of selected split cattle hides. The advantages are their standard diameter and strength and that they can be "shirred", i.e. folded together, in long lengths and used for manual or automatic filling stations without pre-soaking in water.

Synthetic casings

Synthetic casings are made of synthetic thermoplastic materials: Polyamide (PA), Polyethylene (PE), Polypropylene (PP), Polyvinylidenchloride (PVDC) and Polyester (PET). They can be manufactured with tailor-made properties but cannot be used for products which have to undergo drying, ripening and fermentation, such as with dry sausages, since they are impermeable for gases and water vapor.

351. Rounds are 40 m long and are normally readily available where cattle are slaughtered.
352. Lyoner or sausage is a sausage without lining. The recipe originally came from the French city of Lyon - there she is Cervelas called.
353. The beef middles are separated from the "ruffle" (mesenteric fat), flushed out with water, trimmed free of fat, turned inside out, slimed and salted. Beef middles include the "straight" casing (long, not curved part) and are packed in sets each measuring about 17m after salting and composed of 5 pieces.
354. Collagen is an animal tissue fit for human consumption, the thin collagen casings are easy to chew and "edible". They are an alternative to replace natural sheep, goat or thin pig casings.

Glycosaminoglycans

These are aminopolysaccharides found in various forms throughout most animal tissues. Heparin[355] is obtained from intestinal mucosa, mainly from sheep and pigs. It is used commercially as a blood anticoagulant to prevent blood from clotting during operations and during blood dialysis. Derivatives of heparin and similar glycoaminoglycans are used to improve peripheral blood circulation. As a cosmetic heparin (applied topically) is used to reduce bruising and sometimes as a moisturizer.

Suturing material

Catgut has been used by humans for centuries. It is extremely durable and strong,[356] and can be used to suture, to string musical instruments, and to string tennis rackets.

After surgical operation, sutures hold the basic structural elements in their required sites and provide necessary strength. They are characterized as biodegradable or non-biodegradable. The first are used mainly for internal wound closures. The latter are used for closed exposed wounds and are removed when the wound is quite healed. Sutures may be of a natural or synthetic variety.

A catgut[357] suture is a suturing material made from the natural fiber found in the walls of animal intestines that is naturally degraded by the body's own proteolytic enzymes. It is a popular material because it is absorptive and can be sterilized. Historical references to the use of twisted intestinal materials for surgical suture go back to the 2nd century A.D. and its use in musical instruments far longer. The name catgut and its use as suture first appeared in the writings of Claudius Galenus around 50 AD. Sheep have historically been used as a source for catgut, although dogs, cattle, goats, hogs, horses, mules, or donkeys have been used as well. Absorbable sutures can be plain catgut, which usually holds its strength for about 10 days or catgut treated with chromic acid (chromic sutures) that has a slower absorption and keeps its strength for 20 days. Catgut surgical sutures, both chromic and plain, can be used in general closure, ophthalmic, orthopedics, dentistry, gynecology and gastro surgeries.

355. Heparin is a polymer classified as a mucopolysaccharide or a glycosoaminoglycan. It is biosynthesized and stored in mast cells of various mammalian tissues, particularly liver, lung and mucosa. Commercial heparin is chiefly isolated from beef lung or pork intestinal mucosa. See: Linhardt R. J., N. S. Gunay (1999), *Production and chemical processing of low molecular weight Heparins*, Seminar in Thrombosis and Hemostasis, Thieme Medical Publishers, 25 (3), N.Y.

356. Catgut strings are made preferably from the small intestines of sheep. To produce the strings, the guts are cleaned and cut into about 15 m strands before being chemically treated to preserve them. Each string is made up of 15 individual strands which are spun very tightly together. Then they are dried out in a humid room to prevent cracking. It is a painstaking process that takes six weeks from start to finish.

357. Catgut sutures, despite the name, perhaps have nothing to do with cats. The word appears to have come into common use several hundred years ago. Although it would be possible to derive catgut from cats, the yield would not be impressive when compared to that of a larger animal. The origins of the term "catgut" appear to come from "kitgut," a word derived from "kit" for "fiddle." However, since a small cat is also called a Kit, some say this was the evolutionary pathway from "Kat gut" to "Catgut". Kitgut fiddle strings were made from the processed intestines of animals such as sheep, and over time, the word was corrupted into "catgut." Another maintains that the best lamb gut musical strings came from Catagniny, Germany. Top musicians demanded Catagniny Gut, which eventually evolved into "Catgut". Confusion about the term persists to this day.

Catgut, however, can possibly lose its tensile strength, have tainted purity, and be expensive.[358] Collagen was invented to overcome the disadvantages of catgut. The flexor tendons of beef are converted into dispersed fibrils that are extruded and reconstituted to form collagen sutures.

There is debate about whether to continue using catgut in a medical setting, since cotton is usually cheaper and wounds closed with either cotton or synthetic threads (as polyglycolic acid and polyglactin[359]) are less prone to infection. Biosecurity Australia, an agency within the Australian Department of Agriculture Fisheries and Forestry, identifies catgut as a "high TSE risk product" since it is derived from bovine or ovine intestinal tissue.[360] In Europe and Japan, gut sutures have been banned since 2002 due to concerns that they could transmit bovine spongiform encephalopathy (mad-cow disease), even though the herds from which gut is harvested are certified BSE-free. In Italy the Decree of 16 July, 2002 from the Ministry of Health[361] prohibited "the use, importation and placing on the Italian market of medical devices, in the form of absorbable surgical sutures (catgut), obtained from bovine, sheep and goats intestine, as well as the use, importation and placing on the Italian market of medical devices obtained from materials of bovine origin coming from the dura mater, brain and spinal cord".

The use of animal intestines to make thread for sewing footwear and fur clothing has been known from very ancient times.

Musical string

For thousands of years, the choices of musical string materials were few. Usually it was limited to some indigenous material that was suitable to the task. For a long period, catgut was the most common material for harps, lutes, violins, and violas strings, as well as for the surface of older marching snare drums.

No one knows exactly when catgut was first used for musical strings. According to legend, Apollo was the first string maker. One day, when he came across a tortoise, he was inspired to make the first lyre, and he used the poor animal's own intestines for the strings. The first evidence of gut string use dates back to 1823. The architect James Burton Jr. purchased some musical instruments[362] in Thebes (Greece) for his early musical instrument collection.

358. Doctors at the New Orleans Charity Hospital had found that wounds stitched together with "ordinary cotton thread" were less likely to become infected than those sutured with catgut or silk. Another advantage: cotton is not absorbed and will hold when a wound takes a long time to heal—catgut may disappear in a little over a week.

359. Leroux N., E. Bujold (2006), "Impact of chromic catgut versus polyglactin 910 versus fast-absorbing polyglactin 910 sutures for perineal repair: a randomized, controlled trial", *American Journal of Obstetrics and Gynecology*, 194 (6): 1585-90; Kettle C., R. B. Johanson (2010), *Absorbable synthetic versus catgut suture material for perineal repair*, in The Cochrane Library, Issue 2,. Chichester, UK: John Wiley & Sons, Ltd. Search date 1999.

360. Department of Agriculture Fisheries and Forestry Australia, *Measures to address additional TSE concerns with veterinary vaccines and other high risk biologicals*, November 2001.

361. Ministero della Salute, "Misure di protezione nei confronti delle encefalopatie spongiformi trasmissibili relativamente ai dispositivi medici" ("Protective measures with regard to transmissible spongiform encephalopathies in relation to medical devices", Official Gazette of the Italian Republic n. 192 of 17 August, 2002).

362. Some are currently in the British Museum at London.

These interesting relics had been found in a tomb at Thebes. One stringed instrument, when found, "was nearly perfect, and had parts of the catgut of all the four string".[363] An Egyptian harp had gut strings that still made a tone after some two thousand years in storage.[364] Most musical instruments produced today use strings with cores made of other materials, generally steel or synthetic polymer. Catgut strings are still commonly preferred in concert-tension pedal/grand and some lever harps, because they produce a richer, darker sound as well as withstand high tension within low alto, tenor, and high-bass ranges.

Strings for tennis racquet

On the market there are many different types of strings; selecting a string can be challenging as there are hundreds of strings to choose from. Making gut strings from cows requires a complex process. Because of this, gut is the most expensive string on the market.[365] Gut string is very popular among professional players because of its elasticity, tension stability, and liveliness. Because of its high price, however, gut is not recommended for the average recreational player. It is also not very durable and is very sensitive to moisture.

Synthetic gut strings provide different characteristics such as durability, spin, feel, power, etc. They can be made from nylon, polyester and Kevlar, and multifilaments, etc.

In recent years hybrid strings are gaining popularity as more players are looking for a blend of string qualities. By selecting different hybrid combinations of string, players can fine-tune the playability, comfort, durability, liveliness, and control offered by the stringbed. For instance, heavy hitting players can find a good combination of durability and playability with a polyester main string and natural gut or premium synthetic cross string hybrid.

Small intestine submucosa

The mucous membrane is the weakest of the four layers that make up the intestines, and it is removed when processing the raw material. The submucous layer is the strongest and it is always left intact.[366] The muscular and serous layers are removed or left depending upon how tough they are (which is determined by the section of the intestine) and the purpose of the finished product. Intestines are usually washed in the slaughterhouse. The intestine wash water contains valuable mucus that can be recovered, cleaned for pathogen material, concentrated to 150 centipoises, and spray dried.

Porcine small intestine submucosa (SIS)[367] can be implanted into humans to help repair

363. Limbird J. (1836) (Publisher), *The Mirror of Literature, Amusement, and Instruction*, Vol. XXVIII, London, p. 98 (Egyptian Antiquities: Musical Instruments).

364. Wilkinson J. G. (1841), A second series of the Manners and customs of the ancient Egyptians: including their religion, agriculture, & c. Derived from a comparison of the paintings, sculptures, and monuments still existing, with the accounts of ancient authors, Vol. I, J. Murray, London.

365. It seems that it takes about four cow's guts to string an average racquet.

366. Bejjani G. K., J. Zabramski (2007), "Safety and efficacy of the porcine small intestinal submucosa dural substitute: results of a prospective multicenter study and literature review", *Journal of Neurosurgery*, 106 (6): 1028–33; Badylak S., K. Kokini, B. Tullius, A. Simmons-Byrd, R. Morff (2002), "Morphologic study of small intestinal submucosa as a body wall repair device", *Journal of Surgical Research*, 103 (2): 190–202.

367. Zheng M. H., J. Chen, Y. Kirilak, C. Willers, J. Xu, D. Wood (2005), "Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: Possible implica-

everything from eyelids to eardrums. The SIS material, which is not rejected by the human and animal body, signals the patient's own cells to begin to grow and repair tissues. There have been studies regarding this phenomena.

Sandusky Jr. and others[368] implanted a small caliber vascular graft from porcine small intestine submucosa in a canine carotid artery. They later compared it with an autogenous saphenous vein graft that was implanted in the contralateral carotid artery. The results of these studious indicate that the SIS graft is similar to saphenous vein graft in the dog.

Badylak and others[369] tested the use of autogenous small intestinal submucosa (SIS) as a large diameter (10 mm) vascular graft in the infrarenal aorta of 12 dogs. They conclude that autogenous small intestinal submucosa can be successfully used as a large diameter arterial graft in the dog and is worthy of further investigation.

Kropp and others[370] determined the feasibility of promoting urinary bladder regeneration with porcine-derived small intestinal submucosa (SIS). This study further supports the concept of bladder regeneration and suggests that SIS may be a viable material for bladder augmentations.

Alvin B. Rutner and others[371] described their surgical technique and results with implantation of processed porcine small intestine submucosa (SIS) as a pubo-vaginal sling in 152 consecutive female patients with stress urinary incontinence (SUI). They concluded that processed SIS is strong, durable, biocompatible, infection resistant, and gradually replaced by host tissues.

However, there are doubts about the use of SIS on people.[372] The long-term efficacy of pubo-vaginal sling (PVS) procedure with porcine small intestinal submucosa (SIS) implant was retrospectively assessed by Siracusano and others.[373] They concluded that PVS procedure using SIS cannot offer a durable option for the treatment of SUI as reported by the current mini-invasive techniques.

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tions in human implantation", *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 73B (1): 61–67; Dejardin L. M., S. P. Arnoczky, B. J. Ewers, R. C. Haut, R. B. Clarke (2001), *Tissue-Engineered Rotator Cuff Tendon Using Porcine Small Intestine Submucosa. Histologic and Mechanical Evaluation in Dogs*, Presented at the 26th annual meeting of the AOSSM, Sun Valley, Idaho, June 2000 (in *The American Journal of Sports Medicine*, 2001, vol. 29, pp. 175-184); McDevitt C. A., G. M. Wildey, R. M. Cutrone (2003), "Transforming growth factor-β1 in a sterilized tissue derived from the pig small intestine submucosa", *Journal of Biomedical Materials Research Part A*, vol. 67A (2): 637–640.

368. Sandusky Jr. G. E., S. F. Badylak, R. J. Morff, W. D. Johnson, G. Lantz (1992), "Histologic Findings After In Vivo Placement of Small Intestine Submucosal Vascular Grafts and Saphenous Vein Grafts in the Carotid Artery in Dogs", *American Journal of Pathology*, 140 (2): 317-324.

369. Badylak S. F., G. C. Lantz, A. Coffey, L. A. Geddes (2002), "Morphologic study of small intestinal submucosa as a body wall repair device", *Journal of Surgical Research*, 103 (2): 190–202.

370. Kropp B. P., B. L. Eppley, C. D. Prevela, M. K. Rippy, R. C. Harruff, S. F. Badylak, M. C. Adams, R. C. Rink, M. A. Keating (1995), "Experimental assessment of small intestinal submucosa as a bladder wall substitute", *Urology*, 46 (3): 396–400.

371. Rutner A. B., S. R. Levine, J. F. Schmaelzle (2003), "Processed porcine small intestine submucosa as a graft material for pubovaginal slings: durability and results", *Urology*, 62 (5): 805–809.

372. Schaefer M., A. Kaiser, M. Stehr, H. J. Beyer (2013), "Bladder augmentation with small intestinal submucosa leads to unsatisfactory long-term results", *Journal of Pediatric Urology*, published online 17 January 2013.

373. Siracusano S., S. Ciciliato, N. Lampropoulou, A. Cucchi, F. Visalli, R. Talamini (2011), "Porcine small intestinal submucosa implant in pubovaginal sling procedure on 48 consecutive patients: long-term results", *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 158 (2): 350–353.

4.11 Lungs

Pig, calf and lamb lungs, as foods, are mainly used to make stuffing and some types of sausages and processed meats.

In 1963, the first single human lung transplant was performed at the University of Mississippi,[374] but the patient died within days because of renal failure and general debility. Others transplants followed with increasing success.[375]

Heparin can also be extracted from the lungs for use as an anti-coagulant, gangrene preventative, and as meal (as already mentioned above). Heparine is also prepared from bovine lung and bleached during processing. It has been traditionally used as an anticoagulant and binds to antithrombin III, a naturally occurring plasma protease inhibitor, accelerating the rate at which antithrombin III inhibits coagulation proteases.[376]

Aprotinin is another compound obtained from bovine lungs and available on the market as lyophilized powder. The term is typically used when describing the protein derived from bovine lung.

Aprotinin was isolated independently from two laboratories and originally named Bovine Pancreatic Trypsin Inhibitor and Trypsin-kallikrein Inhibitor. It is a protein consisting of 58 amino acids, arranged in a single polypeptide chain that is crosslinked by three disulfide bridges. It is an antifibrinolytic medicine, which prevents excessive blood loss. Aprotinin is a competitive serine protease inhibitor that inhibits trypsin, chymotrypsin, kallikrein and plasmin.[377]

Aprotinin is producted by lungs. The manufacture involves extraction from minced tissue, separation of solids, clarification, ultrafiltration and concentration and fractionation by reverse-phase chromatography. Extration, removal of solids, clarification by alluvial filtration, and ultrafiltration are standards tasks. Following concentration, the final yield volume is about 10 liters from 100 kg of lung.

4.12 Meat and bone meal

Meat and bone meal (MBM) revenue is an important aspect to the profitability of rendering operations and the meat industry in general. Usually destined for disposal, bio-oils and higher value chemical products can be extracted from meat and bone char for use as internal energy or for sale.

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374. Hardy J. D. (1999), "The First Lung Transplant in Man (1963) and the First Heart Transplant in Man (1964)", *Transplantation Proceedings*, 31: 25–29.

375. Arcasoy S. M., R. M. Kotloff (1999), "Lung Transplantation", *New England Journal of Medicine*, 340 (14): 1081–1091.

376. Melo F. R., M. S. Pereira, D. Foguel, P. A. S. Mourão (2004), "Antithrombin-mediated Anticoagulant Activity of Sulfated Polysaccharides. Different Mechanisms for Heparin and Sulfated Galactans", *The Journal of Biological Chemistry*, 279 (20): 20824-20835; Björk l., U. Lindahl (1982), "Mechanism of the anticoagulant action of heparin", *Molecular Cellular Biochemistry*, 48 (3): 161-182; Jordan R., D. Beeler, R. Rosenberg (1979), "Fractionation of low molecular weight heparin species and their interaction with antithrombin", *The Journal of Biological Chemistry*, 254 (8): 2902-2913.

377. Kassell B., M. Laskowski Sr. (1965), "The basic Trypsin inhibitor of bovine pancreas: V. The disulfide linkages", *Biochemical and Biophysical Research Communications*, 20 (4): 463-468; Kassell B. (1970), "Bovine Trypsin-Kallikrein inhibitor (Kunitz Inhibitor, Basic Pancreatic Trypsin Inhibitor, Polyvalent Inhibitor from Bovine Organs)", *Methods in Enzymology*, 19: 844-852.

The most mature and suitable technologies for implementation within the meat processing industry are Pyrolisis,[378] Fluidized bed reactor, Anaerobic digestion, and Co-firing/ Incineration.

Pyrolisis

Pyrolisis is a thermochemical decomposition of organic material at various temperatures (high, low, or medium) in the absence of oxygen. The process is very widespread in the chemical industry: for example, to produce charcoal, activated carbon, methanol, and other chemicals from wood; to convert ethylene dichloride into vinyl chloride to make PVC; to produce coke from coal; to convert biomass into syngas and biochar; to turn waste into safely disposable substances; and for transforming medium-weight hydrocarbons from oil into lighter ones like gasoline.

Pyrolysis typically occurs under pressure and at operating temperatures above 430 °C when organic materials are transformed into gases, small quantities of liquid, and a solid residue containing carbon and ash.

The main potential of this technology is the production of a liquid fuel (bio-oil) suitable for transport and storage. Its advantage of over other methods of energy extraction from MBM is the milder operating conditions, around 500°C instead of 800-900°C for gasification, and the very short processing times compared to the several weeks required for anaerobic digestion. Key to commercial success seems to be the extraction of higher value chemical by-products that occur naturally during the pyrolysis of biomass, in addition to the bio-oil itself.

MBM typically is a fine powder and has low moisture content (about 5%), making it a good feed for pyrolysis. But a basic financial analysis indicates this use of MBM is not yet viable and the process will not be considered while a market for MBM as a animal feed food ingredient remains.

Fluidized bed reactor

Another suitable technology for implementation within the meat processing industry is the fluidized bed reactor. Unfortunately, the small feed size requirements to aid reaction rate may be a problem considering of the energy required for particle size reduction, with the exception of MBM.

Anaerobic digestion

Anaerobic digestion of organic material produces methane and carbon dioxide gases and fertilizer. This process does not deactivate pathogens since the maximum temperature attained in commercial composting is below that required for pathogen inactivation. Consequently it is possible that the presence of "high risk" materials (i.e., brain, spinal cord, etc.) may not be allowed to enter the process stream. MBM may require prior heat treatment (pasteurization) in order to meet further use regulations, and this involves significant additional costs to the anaerobic digestion process.

378. McGlashan S. A. (1997), *Industrial and Energy Uses of Animal By-Products, Past and Future*, Environment and Co-Products Meat and Livestock Australia, Ltd. (http://assets.nationalrenderers.org/essential_rendering_ industrial_and _energy.pdf).

Co-firing/Incineration

Co-firing/Incineration can be used in the production of cement where co-firing/incineration of MBM offers several advantages over other disposal options. This substitution not only provides a method of energy recovery but also reduces net greenhouse gas emissions by replacing coal with a "carbon neutral" fuel. Derived from a biomass, is considered carbon neutral because the carbon released upon combustion was the same absorbed from the atmosphere during the growth of the organism. Another advantage is that the resultant ash is incorporated in the final cement product, which reduces the amount of solid waste ending up in landfills.

MBM inclusion in concrete and asphalt construction composites appear to have some promise. Further study is needed using MBM in construction applications.

4.13 Ovaries

There are many organs and glands in the body of animals: thyroid, adrenal, thymus, testis, ovary glandular, etc. Glands contain hormones, amino acids, vitamins, enzymes, minerals, neurotransmitters, and many others compounds.

Past and modern ealers have used tissue extracts, in addition to herbs and plants, in the fight against disease. For example, desiccated thyroid is still used by many alternative practitioners in the management of hypothyroidism. Many people consume glands, perceiving them to be a source for natural hormones, vitamins, etc. For example, they think that eating brain tissue could improve mental function and eating heart tissue improves heart function, etc. It is known that Thyroid glandular could improve thyroid function in those who are hypothyroid. But one cannot always apply this principle to other glands.

There are no published studies about the safety or effectiveness of bovine ovary extract in humans. Many think that bovine ovary extract stimulates the pituitary gland resulting in an increase in prolactin and growth hormone levels.

Ovary extracts have various activities: ovary extracts may influence estrogen levels. Properties of ovarian extracts have been studied since before World War II.[379] The ovary is made up of many distinct parts: the corpora lutea, the maturing graafian follicles, the interstitial tissue, and the primary, or immature follicles. Progesterone has been obtained from the corpora lutea. The corpora lutea also yields a substance with estrogenic power that is thought to be estrone. Progesterone and estrogen can be extracted from pig ovaries. They may be used to treat reproductive problems in women.

Relaxin, a substance with specific action on pelvic ligaments, is taken from the ovaries of pregnant sows and is often used during childbirth. The effects of ovarian extracts containing relaxin on various types of smooth muscle have been investigated by Miller and others.[380] Administration of the extracts to dogs produced hypotension, bradycardia, inhibition of ureteral motility, and tachyphylaxis.

Many researchers have studied the properties of ovarian extracts. Let us remember some of them. According to Spanel-Borowski[381] ovarian extracts appear to exert influence on

379. Marlow H. W. (1939), "Properties of ovarian extracts", *Endocrinology*, 25 (5): 793-797.
380. Miller J. W., A. Kisley, W. J. Murray (1957), "The Effects of Relaxin-Containing Ovarian Extracts on Various Types of Smooth Muscle", *Journal of Pharmacology and Experimental Therapeutics*, 120 (4): 426-437.
381. Spanel-Borowski K., S. Weis (2007), "Ovarian Extract have an effect on Chemotaxis of Blood Granulocytes. A Study Conducted in Superovulated Golden Hamsters", *Reproduction in Domestic Animals*, 25 (5): 269-276.

chemotactic activity and adherence of blood granulocytes. Heilbrunn and others[382] pointed out the antimitotic and carcinostatic action of ovarian extracts.

4.14 Proteins

For non-food proteins there are many potential applications. Among these we remember the following:

- Protein hydrolysis products
- Proteins for Plastics
- Protein-based adhesives
- Protein surfactant

a) Protein hydrolysis products

Proteins are made up of chains of amino acids linked by peptide bonds and folded in a variety of complex structures. Protein hydrolysis is the breakdown of protein into smaller peptides and free amino acids. Protein hydrolysates, also called peptones, are the result of the hydrolysis process on protein material.

Peptones are obtained from enzyme catalysed hydrolysis and hydrolysates from acid catalysed hydrolysis. Meat peptone is used in biotechnology as a nutrient in vaccine production and in microbiological culture medium. Casein peptone is also used as a culture medium while casein hydrolysates are used as an amino acid supplement (a nutriceutical) and in cosmetics.

As starting material, commonly used protein sources, include meat, casein and whey (milk proteins), gelatin, and soy beans. Hydrolysis of the starting material is accomplished by utilizing acid[383] or enzymatic[384] or microbial[385] hydrolysis.

b) Proteins for plastics

Demand of environmentally friendly plastics manufactured from biodegradable materials is growing continuously.[386] Plastic films can be made from thermoplastic polymers by blowing or casting or molding. Extrusion is used for collagen products: a purified and acidified aqueous suspension is extruded into a coagulating bath. Thermoplastic extrusion is not employed for protein-based films. A cast film is made by laying a solution of the polymer

382. Heilbrunn L. V., W. L. Wilson, T. R. Tosteson, E. Davidson, R. J. Rutman (1957), "The Antimitotic and Carcinostatic Action of Ovarian Extracts", *Biological Bulletin*, 113 (1): 129-134.
383. Adler-Nissen J. (1979), "Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid", *Journal of Agricultural and Food Chemistry*, 27 (6): 1256-1262.
384. Adler-Nissen J. (1976), "Enzymatic hydrolysis of proteins for increased solubility", *Journal of Agricultural and Food Chemistry*, 24 (6): 1090-1093; Adler-Nissen J. (1986), *Enzymatic hydrolysis of food proteins*, London: Elsevier Applied Science Publishing.
385. Ferrero M. A. (2000), *Protein Hydrolysis: Isolation and Characterization of Microbial Proteases*, in John F. Spencer and Alicia L. Ragout Spencer (Edts.), *Food Microbiology Protocols*, Series: Methods in Biotechnology, Vol. 14, Springer Science+Business Media, Secaucus, N. J. (USA): 227-232.
386. Kolybaba M., L.G. Tabil, S. Panigrahi, W. J. Crerar, T. Powell, B. Wang (003), *Biodegradable Polymers: Past, Present, and Future*, presented at the 2003 CSAE/ASAE, Annual Intersectional Meeting, Sponsored by the Red River Section of ASAE, Fargo, North Dakota, USA, October 3-4, 2003.

dissolved in a volatile solvent on a flat surface. Solvent evaporation leaves a polymer film. Forming the polymer relies on a solvent casting that uses water, acidic water, alkaline water, or aqueous ethanol as the solvent (depending on the type of protein).

Currently, the most mature technologies use wheat and cornstarches, soy proteins, and oil-derived esters as raw material. A wide range of proteins have been used to produce edible and/or biodegradable casings and coating for food, pharmaceuticals, and industrial products. For a long time protein films have been used as meat casing. Other uses include collagen casings, gelatin capsules, and microcapsules. Lately, the search for biodegradable polymers for packaging and food wraps has resulted in the growth of research and in a development of activity.[387] However, few commercially produced biodegradable plastics are price competitive with traditional oil-derived plastics such as polyethylene and polystyrene. Moreover, bio-based plastic derived from fermentation processes, i.e. as for protein-based plastics, are generally more expensive than those manufactured via chemical processes. Furthermore, most biodegradable plastics are mechanically inferior to polyethylene and polystyrene, though the properties of protein films can also be modified by cross-linking the protein molecules and modifying the molecular structure using various physical and chemical processes. The hydrophilic nature of protein films implies that they have poor moisture barrier properties, though structural modification or the addition of lipids, waxes and so on can decrease the water vapor transmission rate.[388]

Protein films tend to be brittle, so some plasticizers can be used, such as propylene glycol, glycerol, triethylene glycol, sorbitol, and sucrose, etc. The use of plasticizers tends to decrease film stiffness and tensile strength, while increasing elasticity and permeability.

c) Proteins as adhesives

Protein streams from rendered co-products are well suited to adhesive applications due to the large number of available chemical functionalities associated with the amino acids that are useful for bond formulation. The functional group can then form both physical bonds with the substrate material or can form true chemical bonds with the functional group found on the substrate.

The use of waste protein as a raw material in the manufacture of adhesives for wood composites has been the subject of extensive study in many countries over the past 50 years. Animal protein-based adhesives can be derived from animal blood, although some involve the use of specific proteins primarily selected from collagen and blood albumin.

Mechanisms for using proteins as adhesives are gel penetration (the gel penetrates the pores of substrates), protein-substrate bonding, thermoset encapsulation and high-adhesion protein structures.

Animal-based adhesives have been used since the early 1800s and consumption peaked at about 70,000 tons in the USA in 1948. After World War II, low cost synthetic adhesives quickly infiltrated the market replacing those products from animals that were because technically inferior and more expensive.

387. The state of the art for vegetable and animal protein film applications are reviewed by Gennadios A. (2002) (Edt.), *Protein-based films and coatings*, CRC Press, Florida (USA).

388. Tharanathan R. N. (2003), "Biodegradable films and composite coatings: Past, present, and future", *Trends in Food Science and Technology*, 14 (3): 71-78; Aminabhavi T. M., R. H. Balundgi, P. E. Cassidy (1990), "Review on biodegradable plastics", *Polymer Plastics Technology and Engineering*, 29 (3): 235-262; Fomin V. A. (2001), "Biodegradable polymers, their present state and future prospects", *Progress in Rubber and Plastics Technology*, 17 (3): 186-204.

The largest adhesive market dominated by urea-formaldehyde[389] and phenol-formaldehyde[390] resins is for the production of wood products such as plywood. Consequently, the primary target market is for protein-based adhesive formulations that may act as substitutes for formaldehyde resins is the plywood market, but there are few large-scale uses of waste animal proteins inhibiting development of this market. The costs of transforming waste animal protein into a form that is suitable for use in adhesive formulations makes this use economically attractive. But it is not just a matter of cost. There is also the problem of the low water resistance of protein-based adhesives and resulting accelerated bio-deterioration of the product. Research into cross-linking processes and reactive addition or modification of functional groups may overcome some aspect of the adhesives poor water resistance.

d) Protein surfactants

"Surfactant" is a contraction of the term "surface active agent". This agent is a chemical that is structurally attracted to the interface between two phases of matter. In other word, surfactants are compounds that lower the surface tension of a liquid, the interfacial tension between two liquids, or the surface tension between a liquid and a solid.[391] The surfactants are characterized by their amphipathic[392] structures: one part of the molecule will be lypophobic, while the other part will be lypophillic. Proteins, as already mentioned, are polymers with a lot of amino acids that have a number of functional groups that can interact with two sides of an interface or that can be derivatized with another compound to form an amphipathic structure.[393]

Surfactants are used as wetting agents, foaming and anti-foaming agents, emulsifiers, and dispersion and aggregation agents.

Protein-based surfactants are used in the cosmetic and personal care industry.[394] At present, vegetable derived proteins are almost always preferred in the personal care market. However, surfactant production is dominated by synthetic surfactants made from petroleum.

4.15 Stomach and tripe

Ruminants are herbivores that utilize, in their forestomach (rumen), a symbiotic relationship with microorganisms in order to exploit fibrous feeds as a source of energy and nutrients.[395]

389. Urea-formaldehyde resins are used as adhesives for indoor wood composite products as they are less water-resistant.

390. The phenol-formaldehyde resins are used for outdoor applications for their water resistance and to minimize the effects of formaldehyde emission.

391. See: Rosen M. J., J. T. Kunjappu (2012), *Surfactants and Interfacial Phenomena*, 4th Edition, John Wiley & Sons, Hoboken, New Jersey.

392. Amphiphile from the Greek amphis (both) and philia (love, friendship). It is a term describing a chemical compound possessing both hydrophilic and lipophilic properties. Such a compound is called amphiphilic or amphipathic.

393. Krantz D. D., R. Kagan, S. Lawrence Zipursky (1991), "Amphipathic β Structure of a Leucine-rich Repeat Peptid", *The Journal of Biological Chemistry*, 266 (25): 16801-16807.

394. Nnanna I. A., Jiding Xia (2001), Protein-Based Surfactants - Synthesis, Physicochemical Properties and Applications, CRC Press, Florida (USA).

395. Microbial fermentation of ingested plant materials is a crucial step in the digestion of feed by the host animal. Most microorganisms have different roles in feed digestion and act synergistically to fer-

Ruminant stomachs have four compartments: rumen, reticulum, omasum and an abomasum that corresponds to the omnivore stomach. Stomachs are abundant as slaughterhouse by-product and if they are not used as a waste material can create environmental pollution.

a) Ruminant stomach as food

Tripe is a generic term referring to the stomach of various ruminant animals especially cattle, sheep, goats, and deer. Ruminant animals have multi-chambered stomachs[396], and tripe generally comes from either the rumen or reticulum chambers. In other word, tripe is the culinary term for the stomach tissue, or offal, of most ruminant animals. The rumen and reticulum are those most often used as food. They are generally processed at the place of collection by washing, scalding and bleaching. They are suitable for poaching or braising, and can be used in sausages and processed meat and sometimes can also be sewn to form a casing and stuffed.

Tissues from the rumen are usually quite smooth, and are often known as "flat tripe." The reticulum chamber produces the higher-priced honeycombed varieties, which are appreciated for their texture and for their flavor.

Tissues taken from the omasum or reticulum are frequently known as "book" or "leaf" tripe. Abomasums tissues are often referred to as "green" because they can contain undigested food (grasses, leaves, or shrubbery that the animal ate shortly before it died can often be found in this part of stomach). In the USA and Europe most processed abomasum tissues are set aside for use in pet food, as they are a very cheap means of delivering protein to domestic animals.

The health benefits and nutritive content of stomach tissues vary depending on the type of animal and on the chamber from which they are derived. However, in most cases, the stomach tissues are high in iron, calcium, and zinc and are very low in fat and calories, in addition to being excellent sources of protein, vitamin B_{12}.

A 100 g serving of tripe provides 11.8 g of protein, or 18,15% of the 65 g FDA daily value; 4,2 g of fat, or 6% of the 65 g daily value; and 1.5 g saturated fat, or 7,1% of the 20 g daily value. A 100 g serving of tripe provides 81,1 mg calcium, or 8% of the 1,000 mg FDA daily value. A 100 g serving of tripe provides 1.8 mg of zinc, or 11% of the 15 mg FDA daily value. The selenium content in a 100 g of tripe is 11.9 mcg, or 17 percent of the 70 mcg daily value.[397]

The United States Department of Agriculture (USDA)[398] recognizes two types of tripe: honeycomb (Items No. 726 and 727) and other.

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ment plant carbohydrates and proteins. Microbial populations change with feed type.

396. The cellulose and hemicellulose cannot be digested by mammalian enzymes and require bacteria, protozoa and fungi in order to break down their. The rumen contains that microorganism and enzymes. The broken down material then moves to the omasum which acts as a filter and only enables very small particles to move on to the abomasum while larger particles return to the rumen/reticulum for further break down. The abomasum is the "true" stomach and acts like the monogastric stomach by producing acid and protelytic enzymes.

397. Tyler Herbst S., R. Herbst (2007), *The New Food Lover's Companion*, Barron's Educational Series, Inc., New York.

398. United States Department of Agriculture, *Agricultural Marketing Service Livestock and Seed Program*, "Institutional Meat Purchase Specifications - For Variety Meats and Edible By-Products - Series 700", (1993), Washington, DC 20250.

Item No. 726 - Beef Tripe, Scalded, Bleached (Denuded) - The paunch with or without the "honeycomb" reticulum will be scalded and washed absolutely free of any foreign material and bleached with an FSIS approved bleaching solution. The color may range from white to a light pale yellow. The dark internal lining will be removed.

Item No. 727 - Beef Tripe, Honeycomb, Bleached - The "honeycomb" reticulum will be removed from the paunch by cutting along the seam connecting the two sections of the stomach. The dark internal lining will be removed and the tripe will be scalded and bleached to a creamy white color.

Europeans distinguish between four different types of beef tripe, and use all four.

- Plain Tripe or Rumen. Comes from the first stomach (called the "panes" in French) and it is considered the least desirable tripe amongst tripe fans;
- Honeycomb Tripe or Reticulum (called "réseau" in French) comes from the lower part of the second stomach. Pocket Tripe also comes from the second stomach;
- Book Tripe or Omasum (called "le feuillet" in French, "centopelli" or "fogliolo", or "millefogli" or "bibbia" in Italian) comes from the third stomach;
- Reed Tripe or Abomasum (called "caillette" or "franche-mule" in French, "lampredotto"[399] or "fisarmonica" or "frasame", or "riccia", or "frangiata", or "quaglio", or "riccioletta" in Italian). Comes from the fourth stomach. This is the stomach that rennet is obtained from in calves. In Florence (Italy), they even distinguish 2 separate parts of the fourth stomach: the "spannocchia" and the "gala".

Pig stomachs are composed mainly of smooth muscle and collagenous connective tissue. They are cleaned and scalded to remove the mucosa lining and so are suitable for braising, and sometimes also used as a casing for sausages. They are processed in two ways. If the stomachs are to be incorporated into meat mixes for sausage, they are scalded before further processing, whilst, if they are used as casings, only a small opening is made, through which they are cleaned by flushing with plenty of clean water. Pork tripe is called "trippetta" in Italian and is often considered to be cat food.

b) Rumen and rennet

The rumen is the largest compartment of ruminant animals. It serves as a "fermentation room". It is a complicated microbial systems and one of the most fascinating systems in nature.[400] A wide variety of bacteria, fungi and protozoa act together to bioconvert lignocellulosic plant material into compounds which can be taken up and metabolized by the ruminant. Ruminal content (fluid and feed material) contains about 1000 microorganisms per milliliter, including prokaryotic (bacterial and archaeal) and eukaryotic species.[401]

399. The word "lampredotto" derives from the Italian word "lampreda", for the resemblance that the tripe is thought to have to cooked lamprey eel.

400. Weimer P. J., J. B. Russell, R. E. Muck (2009), "Lessons from the cow: what the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass", *Bioresource Technology*, 100: 5323-5331.

401. Qi M., K. D. Jakober, T. A. McAllister (2010), *Rumen Microbiology*, in R. J. Hudson (Edtr), *Animal and Plant Productivity* - Encyclopedia of Life Support Systems (EOLSS). For more information contact: eolssunesco@gmail.com

When ruminant animals are slaughtered the contents of their rumen can become a viable feed resource and, with appropriate processing, could provide a valuable sources of useful microorganisms.

Sauer and others[402] suggest intensifying the studies of the ruminal microbial ecosystem from an industrial microbiologists point of view in order to make use of this rich source of organisms and enzymes.

Rennet[403] (or Chymosin) is obtained from the stomachs of young mammals, baby cows or pigs living on milk, and is especially valued when it comes from the inner lining of the fourth stomach (abomasum) of milk-fed calves. It is a complex of enzymes,[404] used in cheese-making and one of the first commercially available enzymes used in the food industry.[405]

Natural calf rennet is extracted from the inner mucosa of the abomasum of slaughtered unweaned calves. Of course, these stomach is a by-product of veal production. If rennet is extracted from older calves (grass or grain fed) the rennet contains less or no chymosin but a high level of pepsin and can only be used for particular types of milk and cheeses. Milk-specific rennets are available, such as kid goat rennet for goat's milk and lamb rennet for sheep's milk, as each ruminant produces a special kind of rennet to digest the milk of its own species.

Ancient Egyptians were the first to discover the use of rennet about 6000 years ago.[406] Since they used the dried stomachs of animals as containers for storing liquids, it is supposed that the first cheese was occasionally produced when they stored milk in that container. Those containers could have produced solid chunks (mainly formed by casein) and liquid (whey).

Until 1990, rennet was produced from abomasum, glutinous rice wine,[407] and from various "vegetable" rennet, some of which were made from the microorganism Mucor miehei. Over recent years, the growth in the cheese industry and the scarcity on calf rennet have stimulated the research for milk clotting enzyme from alternative sources.[408] Rennet is produced by genetically engineered bacteria into which the gene for the enzyme has been inserted.

Caseins comprise the main protein component of milk and are secreted as micelles with high concentrations of calcium. They are phosphoproteins that represent the products of four genes equivalent to those that encode the bovine alpha s1, alpha s2, beta, and kappa-

402. M. Sauer, H. Marx, D. Mattanovich (2012), "From rumen to industry", *Microbial Cell Factories*, vol. 11: 121-124.

403. "Rennet" The Columbia Encyclopedia, 6th ed. 2012. Encyclopedia.com. 21 Feb. 2013. (See: htmlhttp:// www.encyclopedia.com/).

404. Rennet contains many enzymes, including a proteolytic enzyme (protease) that coagulates the milk, causing it to separate into solids (curds) and liquid (whey).

405. Fox P. F., P. McSweeney, T. M. Cogan, T. P. Guinee (2004), *Cheese: Major cheese groups*, Academic Press., pp. 2.

406. Neelakantan S., A. K. Mohanty, J. K. Kaushik (1999), "Production and use of microbial enzymes for dairy processing", *Current Science*, 77 (1): 143-148.

407. Jiang T., L. J. Chen, L. Xue, L. S. Chen (2007), "Study on milk-clotting mechanism of rennet-like enzyme from glutinous rice wine: proteolytic property and the cleavage site on kappa-casein", *Journal of Dairy Science*, 90 (7): 3126-33.

408. Escobar J., S. Barnett (1993), "Effect of agitation speed on the synthesis of Mucor miehei acid protease", *Enzyme and Microbial Technology*, 15 (12): 1009-1013; Silveira G. G., G. Monteiro de Oliveira, E. J. Ribeiro, R. Monti, J. Contiero (2005), "Microbial Rennet Produced by Mucor miehei in Solid-State and Submerged Fermentation", *Brazilian Archives of Biology and Technology*, 48 (6): 931-937.

caseins.[409] Fiat and Jollès[410] have studied the structural and physiological aspects of caseins of various origins. They concluded that if, in the future, "some of the discussed active peptides (casein fractions of various origins) cannot be characterized in vivo they can all, nevertheless, be synthesized and used either as food additives or in pharmacology".

4.16 Trachea

Trachea is the scientific name for the windpipe, that is the tube connecting the nose, mouth, and throat to the lungs. Bovine Cartilage Extract from the trachea is a rich source of:

- Natural chondroitin sulphate (>20%);
- Glycosaminoglycans (also known as mucopolysaccharides);
- Collagen (Type ll Collagen).

a) Chondroitin sulphate

Chondroitin sulphate (CS) is a compound[411] that is found in blood, cartilage, etc.[412] around joints in the body. Cartilage is a type of connective tissue that is found in the skeletal systems of many animals.

In 1884, chondroitin sulphate was isolated from cartilage by Krukenberg[413] who described the first preparation of glucaminoglycan, but the nature of its monosaccharides and structure was later described in 1925. In 1940, chondroitin was first identified as a component of cartilage. It gained more popularity in 1998 after publication of the book called "The Arthritis Cure".[414] Since then extensive research has been done to observe the efficacy of chondroitin sulfate, especially in its combination with glucosamine to treat osteoarthritis[415] or as anti-inflammatory.[416]

409. Ginger M. R., M. R. Grigor (1999), "Comparative aspects of milk caseins", *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology*, 124 (2): 133-45.

410. Fiat A. M., P. Jollès (1989), "Caseins of various origins and biologically active casein peptides and oligosaccharides: structural and physiological aspects", *Molecular and Cellular Biochemistry*, 87 (1): 5-30.

411. Chondroitin sulfate is a linear heteropolysaccharide consisting of repeating disaccharide units of glucuronic acid and galactosamine, which is commonly sulfated at C-4 and/or C-6 of galactosamine. chondroitin sulfate is a glycosaminoglycan covalently linked to proteins forming proteoglycans. See: Calabrò A., A. Plaas, R. J. Midura, N. J. Goodstone, L. Rodèn, V. C. Hascall (2000), *Structure and Biosynthesis of Chondroitin Sulphate and Hyaluronan*, in R. V. Iozzo (Edtr), *Proteoglycans: Structure, Biology and Molecular Interactions*, Marcel Dekker, Inc., New York-Basel, pp.5-26.

412. Blood, Bladder, Cartilage, Fibroblasts, Kidney, Myelin, Nerve Cells, Nervous Tissues, Neuron, Placenta, Platelet Prostate, Skin, Spleen, Testes, Trachea.

413. Krukenberg C. F. W. (1884), "Die chemischen Bestandtheile des knorpels", *Zeitschrift fur Biologie*, 20: 207-326. In a paper published in 1891 by Schmiedeberg, the name of the material was referred as Chondroitinschwefelsaure, which was derived from chondros (cartilage).

414. Theodosakis J., B. Adderly, B. Fox (1998), The Arthritis Cure: The Medical Miracle That Can Halt, Reverse, And May Even Cure Osteoarthritis, St. Martin's Griffin Edition, New York.

415. Yves H. (2010), "Advances in the Treatment of Osteoarthritis and the Role of Chondroitin Sulphate - A Review", *Musculoskeletal Review*, 5: 11-17; Kahan A., D. Uebelhart, F. De Vathaire, P. D. Delmas, J. Y. Reginster (2009), "Long-term effects of chondroitins 4 and 6 sulfate on knee osteoarthritis: The study on osteoarthritis progression prevention, a two-year, randomized, double-blind, placebo-controlled trial", *Arthritis & Rheumatism*, 60: 524–533.

416. Ronca F., L. Palmieri, P. Panicucci, G. Ronca (1998), "Anti-inflammatory activity of chondroitin

Chondroitin sulfate is manufactured from animal sources such as cow cartilage. CS molecule represents a heterogeneous population the structure of which varies with source. These polymers are extracted from bovine trachea. Nakano and others[417] have studied an economical method for its extraction from bovine nasal cartilage "without introducing any chemicals except acetic acid used for pH adjustment". Chondroitin sulphate seems to have many applications in pharmaceutical, cosmetic, and food industries.

In pharmaceuticals it has been shown to have chondroprotective[418] and antiatherogenic[419] effects in experimental animals. A chondroitin sulphate-iron complex has been reported as an antianemic agent, in which CS contributes to an increased bioavailability of iron.[420] In eye banks, CS is used to increase storage time of corneas[421] and as a solution for preserving corneas used for transplants and during cataract surgery. In health care medicine applications, CS is used for the treatment of neuralgia, nerve migraine headaches, joint pain, shoulder joint pain, and abdominal pain after surgery. The use of bovine cartilage is promoted as an alternative treatment for cancer. In veterinary use it can be injected into the joints of animals to relieve inflammation.

In cosmetics, because of its moisturizing properties and because it possibly increases elasticity and pliability of skin and has the ability to reinforce skin's intercellular matrix, CS

sulphate", *Osteoarthritis Cartilage*, 6: 14-21; Mason P. (2007), *Dietary Supplements*, Third Edition, Pharmaceutical Press, London-Chicago, pp. 68-70; Egea J., A. G.Garcia, J. Verges, E. Montell, M. G. Lopez (2010), "Antioxidant, antiinflammatory and neuroprotective actions of chondroitin sulfate and proteoglycans", *Osteoarthritis Cartilage*,. 18 (1): S24–S28.

417. Nakano T., N. Ikawa, L. Ozimek (2000), "An economical method to extract chondroitin sulphate-peptide from bovine nasal cartilage", *Canadian Agricultural Engineering*, 42: 205-208.

418. Kalbhen D. A. (1983), "Experimental confirmation of the antiarthritic activity of glycosaminoglycan polysulfate", *Zeitschrift für Rheumatologie*, 42: 178–84; Brennan J. J., F. X. Aherne, T. Nakano (1987), "Effects of glycosaminoglycan polysulfate treatment on soundness, hyaluronic acid content of synovial fluid and proteoglycan aggregate in articular cartilage of lame boars", *Canadian Journal of Veterinary Research*, 51: 394–8; Dean D. D., O. E. Mung, I. Rodriquez, M. R. Carreno, S. Morales, A. Agudez, M. E. Madan, R. D. Altman, M. Annefeld, D. S. Howell (1991), "Amelioration of lapine osteoarthritis by treatment with glycosaminoglycan-peptide association complex (Rumalon)", *Arthritis and Rheumatism*, 34: 304-313.

419. Morrison L. M., N. L. Enrick (1973), "Coronary Heart Disease: Reduction of Death Rate By Chondroitin Sulfate a", *Angiology*, 24 (5): 269-287; Matsushima, T., Y. Nakashima, M. Sugano, H. Tasaki, A. Kuroiwa, O. Koide (1987), "Suppression of atherogenesis in hypercholesterolemic rabbits by chondroitin-6-sulfate", *Artery*, 14 (6): 316-337; Kevin Jon W. (2001), "Arterial wall chondroitin sulfate proteoglycans: diverse molecules with distinct roles in lipoprotein retention and atherogenesis", *Current Opinion in Lipidology*, 12 (5): 477-487.

420. Fochi F., M. Ciampini, G. Ceccarelli (1985), "Efficacy of iron therapy: a comparative evaluation of four iron preparations administered to anaemic pregnant women", *The Journal of International Medical Research*, 13 (1): 1-11; Barone D., L. Orlando, E. Vigna, S. Baroni, A. M. Borghi (1988), "Ferric chondroitin 6-sulfate (Condrofer): a new potent antianaemic agent with a favourable pharmacokinetic profile", *Drugs under Experimental and Clinical Research*, 14 (1): 1-14.

421. Keates R. H., B. Rabin (1988), "Extending corneal storage with 2.5% chondroitin sulfate (K-Sol)", *Ophthalmic Surgery*, 19 (11): 817-820; Lass J. H., W. J. Reinhart, D. L. Skelnik, W. E. Bruner, R. P. Shockley, J. Y. Park, D. L. Hom, R. L. Lindstrom (1990), "An in vitro and clinical comparison of corneal storage with chondroitin sulfate corneal storage medium with and without dextran", *Ophthalmology*, 97 (1): 96-103; Tachibana A., M. Sawa (2002), "Development of novel corneal storage medium: first report. Examinations of rabbit cornea", *Japanese Journal of Ophthalmology*, 46 (4): 377-83.

is used in eye lotions, in preparations for the preservation of eyesight, in eye drops for dry eyes, and in shampoos and skin creams.[422]

In the food industries it is used to prepare mayonnaise and dressings,[423] as a nutraceutical[424] in health food drinks (CS is approved by the FDA for these uses).[425]

Chondroitin sulfate is often sold in combination products that also contain glucosamine sulfate. So far, there is no evidence that the combination products work any better than either chondroitin sulfate or glucosamine sulfate alone.

Some doubt on the effectiveness of CS in all the above-mentioned applications was expressed by Henrotin and others[426]: "Despite the moderate effects of CS on pain and function, CS is an interesting product for the management of knee osteoarthritis. Clinical evidence is in favor of a slow-acting effect on symptoms in moderate knee osteoarthritis. CS is recommended by the most popular guidelines. Its safety profile is surely one of its main benefits for the treatment of aging patient with some comorbidity".

However, there isn't enough information to know if chondroitin sulfate is effective for other conditions for which people use it: heart disease, osteoporosis (weak bones), and high cholesterol. Wandel and others[427] in their research to determine the effect of glucosamine, chondroitin, or the two in combination on joint pain and on radiological progression of disease in osteoarthritis of the hip or knee conclude that "Compared with placebo, glucosamine, chondroitin, and their combination do not reduce joint pain or have an impact on narrowing of joint space. Health authorities and health insurers should not cover the costs of these preparations, and new prescriptions to patients who have not received treatment should be discouraged". Clegg and others[428] confirm the ineffectiveness of glucosamine and chondroitin sulfate alone or in combination for the treatment of osteoporosis because they do not reduce pain effectively in patients with osteoarthritis of the knee. Exploratory analyses suggest that the combination of glucosamine and chondroitin sulfate may be effective in the patients with moderate to severe knee pain.

422. Nakamura M., M. Hikida, T. Nakano (1992), "Concentration and molecular weight dependency of rabbit corneal epithelial wound healing on hyaluronan", *Current eye Research*, 11 (10): 981-986.
423. Yabe Y., T. Ninomiya, H. Kashiwaba, T. Tatsuno, T. Okada (1987), "Determination of sodium chondroitin sulfate added in foods", *Journal of the Food Hygienic Society of Japan*, 28 (1): 13–18; Hamano T., Y. Mitsuhashi, N. Acki, S. Yamamoto (1989), "High-performance liquid chromatographic assay of chondroitin sulphate in food products", *Analyst*, 114 (8): 891–893.
424. Kalra E. K. (2003), "Nutraceutical – definition and introduction", *AAPS PharmSci* (American Association of Pharmaceutical Scientists), 5 (3): 27-28.
425. Tallon M. J. (2007), *Key Trends in Nutraceutical Food and Drinks*, Business Insights Ltd., Nottinghamshire (UK), p. 110.
426. Henrotin Y., M. Mathy, C. Sanchez, C. Lambert (2010), "Chondroitin Sulfate in the Treatment of Osteoarthritis: From in Vitro Studies to Clinical Recommendations", *Therapeutic Advances in Musculoskeletal Disease*, 2 (6): 335–348.
427. Wandel S., P. Jüni, B. Tendal, E. Nüesch, P. M. Villiger, N. J. Welton, S. Reichenbach, S. Trelle (2010), "Effects of glucosamine, chondroitin, or placebo in patients with osteoarthritis of hip or knee: network meta-analysis", *British Medical Journal*, 341: c4675
428. Clegg D. O. and others (2006), "Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis", *The New England Journal of Medicine*, 354 (8): 795-808.

According to Lauder[429] "while the safety of CS is not presently in doubt, poor quality finished products have the potential to compromise clinical and lab-based studies and will fail to give consumers all of the benefits available". In conclusion Volpi's[430] position on "a need for specific and accurate analytical procedures, which should be enforced to confirm purity and label claims both for raw materials and finished chondroitin sulfate products, and also to govern the origin of ingredients" seems to be correct.

429. Lauder R. M. (2009), "Chondroitin sulphate: A complex molecule with potential impacts on a wide range of biological systems", *Complementary Therapies in Medicine*, 17 (1): 56–62.
430. Volpi N. (2009), "Quality of different chondroitin sulfate preparations in relation to their therapeutic activity", *Journal of Pharmacy and Pharmacology,* 61 (10): 1271-80.

PART II

Treatment and legislation on ABPs and waste

5. Collection and treatment plants for ABPs

It has already been mentioned that wastes typically arise from food processing and manufacturing plants, distribution premises, food markets, wholesale and retail outlets, and catering facilities (including household kitchens). The European Community has issued a number of directives for waste management.

Landfilling of bio-waste is addressed in the Landfill Directive[431] that requires the diversion of biodegradable municipal waste from landfills. The Industrial Emissions Directive[432] lays down the main principles for the permitting and control of waste treatment installations. The incineration of bio-waste is regulated in the Waste Incineration Directive,[433] while the health rules for composting and biogas plants that treat animal by-products are laid down in the ABPs Regulation.[434]

Since the EU banned the use of rendered animal proteins from the feed chain in 1999, the costs for the treatment of ABPs have increased considerably. Since that EU ABP-Regulation, new opportunities for the utilization of slaughterhouse wastes were opened. For example, in the agri-industrial sector some EU biogas plants[435] are now using pasteurized ABP as co-substrates together with manure, rumen content, and other energy crops (blood, minced hind gut, fat, etc).

There are many premises that generate, store, use, handle and dispose of animal by-products, as set out below:

5.1 - Rendering plants: handling and storage plants;

5.2 - Incinerators/Co-incineration Plants;

5.3 - Landfill;

5.4 - Anaerobic digestion (biogas) plants;

5.5 - Composting;

5.6 - Petfood plants.

5.1 Rendering plant: handling and storage plants (previously known as intermediate plants)

Not all ABP are sent to rendering plants. As already said, meat rich by-products like lungs, liver and hearts are often sold to wet pet food plants and to other utilizations. Ren-

431. Council Directive 1999/31/EC of 26/4/99 on the landfill of waste (Official Journal of the European Communities L 182 of 16.7.99).

432. Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control) (Official Journal of the European Union L 334 of 17.12.2010).

433. Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste (Official Journal of the European Union L 332 of 28.12.2000).

434. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (Official Journal of the European Union L 300 of 14.11.2009).

435. Kirchmayr R., R. Scherzer, D. L. Baggesen, R. Braun, A. Wellinger (2003), Animal by-Products and Anaerobic Digestion - Requirements of the European Regulation (EC) No 1774/2002, IEA Bioenergy, Task 37 - Energy from Biogas and Landfill Gas - In Cooperation with BIOEXELL - Biogas Center of Excellence.

dering is a process which involves cooking animal carcasses or ABPs at high temperature, sometimes under pressure, allowing water to be removed and tallow to be separated from the proteinaceous material. Rendering reduces the volume of disposable material.

The process involves the crushing and grinding of ABP, followed by heat treatment to reduce the moisture content and kill micro-organisms. Heating separates the tallow from the bone and protein. The first can be used as a fuel for the process. The protein and bone are made into meat and bone meal. In research Ramirez and others[436] determined that the weighted average proportion of thermal process energy derived from tallow for six plants in UK, between 2006 and 2008, was 76% and the remaining 24% being derived from natural gas.

Separation of materials is vital to ensure that the risks associated with ABPs are effectively controlled. This includes keeping raw feedstock separate from the processed final product and also allows separation of by-product categories . Procedures must be followed to ensure categories of animal by-products are kept separate from each other at all times in order to minimize the risk of cross-contamination.

In addition, to prevent by-pass of material within facilities, p rocedures should be adopted to control the movement of personnel between areas. . Color coded protective clothing ensures separation between personnel working in different areas (clean and unclean). Procedures must also be adopted to control by-pass risks in communally used areas such as lavatories, changing rooms, and canteens. All staff working on the premises must be adequately trained for their job.

The method of required processing will depend on the Category and nature of the raw material being processed. All rendering plants would contain at least the following process steps:

1. Reception: where the incoming ABPs enter the facility, usuallya form of double door , that provides an odor lock to prevent the release of odors during unloading operations;
2. Identification of biological hazards: the biological hazards (human and animal health) should be identified in view of origin of the raw material;
3. Storage and handling: ABPs are stored, usually in lidded hoppers, for the shortest possible time. Usually ABP boxes are identified by distinctive colors, depending on ABP Category, in accordance with Regulation 1069/2009 of the European Parliament. To comply with ABPs regulations, the raw material must be accompanied by official records of its kind, when received, and when processed;
4. Size reduction: before being passed into the rendering process the ABPs need to be pulpedin order to ensure that all parts of the rendered materials receive sufficient treatment. By mashing a uniform particle size is achieved that equally distributes raw mass. Processing: the crushed ABPs are heated in the rendering plant, where a defined temperature and residence time allow sterilization and the degradation of cellular links for the release of fat;

436. Ramirez A. D., A. Humphries, S. L. Woodgate, R. W. Wilkinson (2012), The potential for energy self-sufficiency in the United Kingdom rendering industry, in Brebbia C. A., S. S. Zubir (Edtrs), *Management of Natural Resources, Sustainable Development and Ecological Hazards*, WIT Press, Southampton (UK), pp. 483-94.

5. Separation of the solid/liquid fraction: the rendering mass is put into the decanters where the liquid fraction is separated from the solid fraction. The solid fraction contains the protein mass of tissue, while the liquid fraction contains fat and water;
6. Separation of the fat: after leaving the decanter, the liquid fraction is lead to an intermediate storage container where the components are separated by a centrifugal separator. That centrifugal force separates the liquid fraction into fats, wastewater, and sludge;
7. Storage of processed materials: meal and fat manufactured by rendering are stored pending users. After treatment the final fat can be used for biodiesel production or can fuel a combustion unit;
8. Washing and cleaning: the plant building, processing equipment, transportation vehicles, and ABP delivery equipment require regular washing and cleaning;
9. Treatment of odorous emissions: because rendering produces odors, plants must have extraction equipment for effective odor abatement;
10. Effluent treatment: all effluent produced must be treated in an approved process on site or sent off site for treatment according to the laws.

As we will see later, Annex IV chapter III of the EU Commission Regulation provides detail of the seven standard processing methods including time, temperature and particle size requirements.

5.2 Incinerators/Co-incineration Plants

In the European Union, incinerators and co-incinerators burning ABPs must be operated in accordance with "Waste Incineration Directive (Directive 2000/76/EC)", "Regulation (EC) No 1069/2009", and "Commission Regulation (EU) No 142/2011 of 25 February 2011"[437] (as amended by Commission Regulation (EU) No 749/2011 of 29 July 2011[438]), which require the disposal or recovery of the resulting ash to be carried out in accordance with environmental legislation. To minimize risk to animal health, Regulation 142/2011 requires that animals are prevented from having access to the ash.

Ash from the incineration or co-incineration of ABPs is subject to waste controls and is normally disposed of in a permitted landfill. Where the feedstock to the incinerator and co-incineration plant is restricted to either animal carcasses or poultry litter only, the completely combusted ash can be spread on land to provide nutrients.

Annex III Section 2 of the EU Commission Regulation states: Operating conditions -Incineration or co-incineration plants will be designed, equipped, built and operated in such a way that the exhaust gas, resulting from the process, is held even under the most unfavourable conditions, at a minimum of 850°C for 2 seconds or 1100°C for 0.2 seconds, as measured at a representative point of the chamber, where the incineration or the co-incineration is carried out. The temperature of a minimum of 850°C for 2 seconds is often achieved using a secondary chamber. The gas temperature in the secondary chamber must be achieved before by-product incineration begins.

437. Commission Regulation (EU) No 142/2011 of 25 February 2011, "implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive", Official Journal of the European Union, L 54 on 26.02.2011.
438. Official Journal No L 198 on 30.7.2011.

Incineration and combustion plants for organic material, such as waste with energy recovery, are common. All new plants in OECD countries must meet strict emission standards, including those on nitrogen oxides (NOx), sulphur dioxide (SO2), heavy metals, and dioxins. Incineration of ABPs must take place at an approved plant. With or without energy generation, incineration of ABPs must be approved under the "Directive on the Incineration of Waste 2000/76 /EC" . Among the many provisions the following are important to recall. (We will revisit these subjects in the next section).

In order to prevent the contamination of foodstuffs with pathogenic agents, establishments or plants processing animal by-products should operate on a separate site from slaughterhouses or other establishments in which foodstuffs are processed. (Commission Regulation (EU) No 142/2011, p. 14, page 5).

For the promotion of science and research and to ensure the best possible use of animal by-products and of derived products in the diagnosis of human or animal diseases, the competent authority should be authorized to lay down conditions for samples of such materials for research, educational and diagnostic purposes. (Commission Regulation (EU) No 142/2011, p. 19, page 6)

Pursuant to Regulation (EC) No 1069/2009, operators are to ensure that animal by-products and derived products are traceable at all stages of the chain of manufacturing, use and disposal, so as to avoid unnecessary disruptions of the internal market in the case of events which are linked to actual or potential risks to public or animal health. Traceability should therefore not only be ensured by operators generating, collecting or transporting animal by-products, but also by operators disposing of animal by-products or derived products, by incineration, co-incineration or landfilling. (Commission Regulation (EU) No 142/2011, p. 22, page 7)

Certain imported materials for the production of pet food should be handled and used under conditions that are appropriate to the risk that such materials may pose. In particular, provision should be made for their safe channeling to establishments or plants of destination where such materials, as well as Category 3 material, are incorporated into pet food. (Commission Regulation (EU) No 142/2011, p. 28, page 8)

Apiculture by-products which are to be placed on the market should be free of certain diseases to which bees are susceptible. These diseases are listed in Council Directive 92/65/EEC of 13 July 1992 which establishes animal health requirements governing trade in and imports into the community of animals. Semen, ova and embryos not subject to animal health requirements laid down in specific Community rules are referred to in Annex A (I) to Directive 90/425/EEC (Commission Regulation (EU) No 142/2011, p. 34, page 11).

If a premises houses which has livestock and only by-products arising on that premise are incinerated then there must be physical and operational separation such that the livestock cannot gain access to the incinerator and there is no risk of passive transfer of by-products to livestock by personnel or equipment. This will require dedicated equipment and clothing for use when operating the incinerator or cleansing and disinfection prior to use associated with livestock (Commission Regulation (EU) No 142/2011, Annex III, Chapter I, Section I).

If an incinerator will be used to incinerate by-products from more than one premise, it must be located on a site that has no livestock: a separate premise. For the location to be considered a separate premise it must, as a minimum, have a defined secure boundary and dedicated entrance . It cannot be incorporated into , or be a part of, i a livestock premise.

It may be possible to approve the use of a mobile incinerator[439] at specified locations which otherwise meet the regulatory requirements of approval for a static incinerator. In such cases an agreed procedure for the cleansing and disinfection of all mobile equipment prior to movement to a new location will be a condition of any approval issued.

Article 24 of the "Approval of establishments or plants" (Regulation (EC) No 1069/2009) establishes that operators will ensure that establishments or plants under their control are approved by competent authority, where such establishments or plants carry out disposal, as waste, by incineration or co-incineration of animal by-products and derived products, excluding establishments or plants which have a permit to operate in accordance with Directive 2000/76/EC.

This applies to most incinerators on farms, at hunt kennels and knackers' yards, and at pet crematoria. Those incinerator plants that burn "processed products" (e.g. meat and bone meal, tallow), catering waste or material other than animal carcasses/parts of carcasses must meet Directive 2000/76/EC requirements.

Regulation (EC) No 142/2011 (Chapter III) permits low capacity (< 50kg/hour) incinerator plants, that are equipped with an auxiliary burner, to burn the following Specified Risk Material: Category 1 materials referred to in Article 8 (b), (e) and (f), Category 2 materials referred to in Article 9 or Category 3 materials referred to in Article 10 of the Regulation (EC) No 1069/2009, that we will see ahead.

5.3 Landfill

Nowadays the production of methane from biodegradable waste decomposing in landfills constitutes a threat to the environment. The EU Landfill Directive (1999/31/EC) has obliged Member States to gradually reduce the amount of biodegradable waste that they add to landfills from 1995 levels until 2016 (for some countries till 2020).

The Landfill Directive does not prescribe specific treatment options for the diverted waste. The benefits of proper bio-waste management, besides avoided emissions of methane and carbon dioxide, would be the production of compost that contributes to enhanced soil quality and methane that is a valuable source of energy. However, Member States are often inclined not to opt for composting or bio-gas production but for the easiest and cheapest option, which is incineration or landfilling.

5.4 Anaerobic digestion

In recent decades soaring globalization, large-scale livestock production, and an escalation in epizootic diseases, has increased the need for biosecurityso that the transmission of disease to the food chain can be minimize. The potential negative environmental impact of biological waste, and waste from ABPs, can be minimized by recycling them into plant nutrients (compost or fertilizers) or converting them into biogas. Since biological waste and/or ABPs can inadvertently spread infectious diseases, obtaining general acceptance for hygienically safe end-products is needed.

There is no doubt that landfilling is the worst waste management option for bio-waste and ABPs. To encourage the management of biodegradable waste diversion from landfills, there seems to be several environmentally favorable options. While a waste management

439. Commission Regulation (EU) No 142/2011, Annex I, pag. 36.

hierarchy applies to the management of bio-waste, in specific cases there may be justification for departures since the environmental balance of the various options available for the management of this waste depends on a number of local factors, i. e. on collection systems, waste composition and quality, climatic conditions, and the potential of use of various waste-derived products such as electricity, heat, methane-rich gas or compost. Therefore, national strategies for the management of this waste should be based on a structured and comprehensive approach (e.g. Life Cycle Assessment, Life Cycle Thinking, ecc.). Anaerobic digestion and composting treatment seem the suitable treatment methods that can combine biosecurity aspects with environmental, economic and nutrient recycling aspects, in order to create a beneficial whole-farm approach.[440] Composting and biogas plants may also be approved by an alternative method set out in Regulation 208/2006. Under this Regulation, approvals are issued according to satisfactory demonstration of sufficient pathogen kill in the treatment process.

As already pointed, in the European Union the landfilling of bio-waste is regulated in the Landfill Directive (1999/31/EC), while the rules for composting and biogas plants that treat animal by-products are regulated in the ABPs Regulation.

It is necessary to remember some definitions of terms.

"Organic fertilizers" can be defined as materials of animal origin used to maintain or improve the physical makeup, chemical properties, and biological activities of soils and/or plant nutrition. Organic fertilizers contain derived products or processed animal proteins (PAP) such as bloodmeal, feathermeal, fishmeal, former foodstuffs and meat and bone meal;

"Soil improvers" or "compost" can be defined as materials of vegetal and/or animal origin used to maintain or improve the physical and biological activities of soils. They cannot be defined "fertilizers";

"Bio-waste" can be defined as food and kitchen waste from households, restaurants, caterers and retail premises; comparable waste from food processing plants; waste from ABPs or processing ABPs; and biodegradable garden and park waste. It does not include agricultural residues or forestry, manure, sewage sludge and by-products of food production that never become waste;

According to the European Union, "Fertilizer" means material of which the main function is to provide nutrients for plants[441]. That definition was absorbed by the Italian law.[442] The "Concimi" (Fertilizers) are divided into «fertilizers CE» e «national fertilizers», whose types and characteristics are reported respectively in Annex I of the Regulation (CE) n. 2003/2003 and Article 2 of Italian Legislative Decree 2010, 29 April, n.75;

"Soil improvers" (ammendanti): materials to be added to the soil in situ, primarily to maintain or improve its physical and/or chemical and/or biological activity (Article 2, Italian Legislative Decree 2010, 29 April, n.75).

440. Albihna A., B. Vinnerås (2007), "Biosecurity and arable use of manure and biowaste — Treatment alternatives", *Livestock Science*, 112 (3): 232–239.

441. Article 2 of the Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003, relating to fertilizers (Official Journal of the European Union, L 304 on 21.11.2003).

442. Article 2 of the Italian Legislative Decree n.75, 2010, 29 April.

Biogas plants

Biogas is a biofuel. Generally biogas refers to the gas produced from organic matter as it is broken down by biological means, in the absence of oxygen.[443] Therefore, biogas can be produced, using anaerobic digesters and by the digestion or fermentation of biodegradable materials such as biomass, food waste, manure, sewage sludge, ABPs, municipal waste, green waste and energy crops such as cereals silage. Produced biogas is comprised primarily of methane (about 58-60%) and carbon dioxide (about 38-42%) and may have small amounts of moisture, nitrogen, hydrogen sulphide, and siloxanes.[444] Natural gas, as known, is composed of about 97% methane. Technologies such as pressure swing absorption and water scrubbing are used to remove CO_2 from the biogas stream, converting it to renewable natural gas. The methane can be used for heating, production of electricity, and many other machines that use an internal combustion engine.

Biogas plants are systems that use a bacteriological process called anaerobic digestion to convert organic waste into biogas.[445] According to Commission Regulation (EU) No 142/2011, ANNEX I, "biogas plant means a plant in which animal by-products or derived products are at least part of the material which is submitted to biological degradation under anaerobic conditions".

Usually, the biogas plant consists of two components: a fermentation tank or digester and a gasholder. The first is a cube-shaped or cylindrical waterproof container with an inlet into which the fermentable mixture is introduced as a liquid slurry or in solid materials form. Bacteria within the digester tank breaks down the waste and, as it decomposes, gases. Through a pressurized system, the gasholder conducts the flow of these gases upward into a hole that is normally an airproof container that cuts off air to the digester and collects the generated gas.[446] A solid residue (digestate), similar but not identical to compost, and a liquid liquor remain. The digestate can be separated out, composted, and sold .[447] The liquor can be used as a fertilizer.

Several anaerobic digester technologies exist. Each is designed to process specific waste streams. Nowadays, there are two main categories of anaerobic digesters: liquid digesters and solid digesters. The first are systems in which the substrate inside the digester is adequately fluid to be pumped (less than 15% dry matter). Solid digesters are systems where the material inside the digester remains solid and is expelled in a solid form. They may run in batches or continuously or semi-continuously.[448]

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443. Torien D. F., W. H. J. Hattingh, J. P. Kotze, P. G. Thiel, W. A. Pretorius, G. G. Cillie, M. R, Henzen, G. J. Stander, R. D. Baillie (1969), "Anaerobic Digestion - Review Paper", *Water Research, Pergamon Press*, 3: 385-416.

444. Wolfe R.S. (1971), "Microbial Formation of Methane", *Advances in Microbial Physiology*, 6:107-146.

445. Gunaseelan V. N. (1997), "Anaerobic digestion of biomass for methane production: a review", *Biomass and Bioenergy*, 13:83-114.

446. Biswas T. D. (1977), "Biogas Plants: Prospects and Limitations", *Invention Intelligence*, 12: 71-78.

447. The value of digestates as fertilizers depends on the make-up of the digestate, and its classification in waste terms. When harmful inorganic substances (e.g. heavy metals), organic substances (e.g. medicinals, PAHs, PCBs, PCDDs, etc.) or injurious weed seeds are present, land application of the digestate may not be possible.

448. Alvarez R., G. Liden (2008), "Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste", *Renewable Energy*, 33 (4), 726-734.

For decades anaerobic digestion has been used for wastewater treatment and for stabilization and volume reduction of sewage sludge, animal manure and organic wastes digestion. Experience has shown that the spreading of digested material of this wastewater type has not caused health problems.When correct processing methods are followed, anaerobic digestion provides a successful method for accessing the energy and nutrient content contained in organic material. Consequently, the utilization of anaerobic digestion for organic waste management permits a significant movement up the waste hierarchy over other management methods.

The value of digestates as fertilizers depends on the make-up of the digestate and its classification in waste terms. With correct storage and application methods and sound agricultural practices, the risk of volatilization and runoff of nutrients of digested material can be greatly reduced compared to risks of storage and application of untreated organic waste and manure.

Recently anaerobic digestion has received increasing attention as a mainstream energy conversion process because it is based on renewable agricultural biomass (energy crops), as well as on co-digestion of various industrial by-products, including animal by-products and wastes.[449] By converting waste into energy, biogas plants reduce odors and pathogens, produce an enhanced fertilizer and reduce greenhouse gas emissions.

Worldwide, Germany is the market leader in this field, with over 4,000 on-farm anaerobic digesters generating more than 1,200 MW of clean power (German Biogas Association, www.biogas.org).

Sami Luste and other[450] have studied the effect of five pre-treatments (thermal, ultrasound, acid, base and bacterial product) on hydrolysis and methane production potentials of four by-products from meat-processing industry. In batch experiments, thermal treatment increased methane production potential of drumsieve waste, acid of grease trap sludge and all pre-treatments of dissolved air flotation sludge. However, with all other pre-treatments, methane production potential was decreased compared to untreated materials, apparently due to inhibition by hydrolysis products and/or possible re-crystallization of some compounds.

5.5 Composting

Natural composting, or biological decomposition, began with the first dead plants on earth and has been going on ever since.[451] As vegetation falls to the ground, it slowly decays, providing minerals and needed nutrients for plants, animals, and microorganisms.

Compost is organic material that can be used as a soil amendment or as a medium to grow plants. Mature compost is a stable material with a content called humus that is dark brown or black and has a soil-like, earthy smell. It is created by combining organic wastes (e.g., yard trimmings, food wastes, manures, etc.) in proper ratios into piles, rows, or vessels.

449. Braun R., R. Kirchmayr (2003), *Implementation Stages of Directive EC 1774/2002 on Animal Byproducts*, in T. Al Seadi and J. Bo Holm-Nielsen (Edtrs), *The Future of Biogas in Europe II -European Biogas Workshop*, October 2-4, 2003, University of Southern Denmark Esbjerg/Denmark.

450. Luste S., S. Luostarinen, M. Sillanpää (2009), "Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry", *Journal of Hazardous Materials*, 164 (1): 247–255.

451. Garland G. A., T. A. Grist, R. E. Green (1995), "The compost story: from soil enrichment to pollution remediation", *Biocycle*, 36 (10): 53-56.

Composting benefits are the following:

- Soil conditioner. Using compost, rich humus for lawns and gardens are created and the compost enrichment helps retain moisture in the soil.
- Recycles kitchen and yard waste. Composting can convert as much as 30% of household waste into soil conditioner and growing mediums.
- Introduces beneficial organisms to the soil. Microscopic organisms in compost help aerate the soil, break down organic material for plant use and ward off plant disease.
- Good for the environment.
- Reduces landfill waste. Most landfills in Europe are quickly filling up; many have already closed down.

In a composting plant animal by-products, or derived products , makeup at least part of the material submitted to biological degradation under aerobic conditions.[452]

It is best not to compost fish scraps (they will attract pests), perennial weeds (they can be spread with the compost). or diseased plants. Pet manures are not included in compost that will be used on food crops. Peach peels, banana peels, and orange rinds may contain pesticide residue; black walnut leaves should not be composted since they can contain toxins. Sawdust may be added to the compost, if it is clean and contains with no lubricating oil residue from cutting equipment.

A new compost technology, known as compost bioremediation, is currently being used to restore contaminated soils, manage stormwater, control odors, and degrade volatile organic compounds.[453]

5.6 Pet food plants

Dogs and cats are carnivores, and therefore consume meat-based diet. The protein used in pet food comes from a variety of sources. When cattle, swine, chickens, lambs, etc., are slaughtered, lean muscle tissue is trimmed away from the carcass for human consumption. About 50% of the weight of every slaughter house animal does not directed to human food. Whatever remains of the carcass (ABPs) can be used in pet food, animal feed, fertilizer, and for others uses. Using Category 3 materials[454] of Regulation (EC) No 1069/2009, which are

452. Annex I - Definitions as referred to in article 2 of Commission Regulation (EU) No 142/2011.

453. To know more about see: United States Environmental Protection Agency, *Innovative Uses of Compost Bioremediation and Pollution Prevention*, Solid Waste and Emergency Response (5306W), EPA530-F-97-042 October 1997 (www.epa.gov); Buyuksonmez F., R. Rynk, T. F. Hess, E. Bechinski (1999), "Occurrence, Degradation, and Fate of Pesticides During Composting Part I: Composting, Pesticides, and Pesticide Degradation", *Compost Science and Utilization*, 7 (4), 66-82; Buyuksonmez F., R. Rynk, T. F. Hess, E. Bechinski (2000), "Occurrence, Degradation, and Fate of Pesticides During Composting, Part II: Occurrence and Fate of Pesticides in Compost and Composting Systems", *Compost Science and Utilization*, 8 (1), 61-81; Buyuksonmez F., S. Sekeroglu (2005), "Presence of pharmaceuticals and personal care products (PPCPs) in biosolids and their degradation during composting", *Journal of Residuals Science & Technology*, 2 (1), 31-40.

454. As we will see ahead, briefly they are: a) carcases and parts of animals slaughtered or, in the case of game, bodies or parts of animals killed, and which are not intended for human consumption for commercial reasons; b) carcases and the parts originating either from animals that have been slaughtered in a slaughterhouse and were considered fit for human consumption (heads of poultry, hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus

materials from healthy animals that would be fit for human consumption but are not consumed for reasons of culture and customer choice are forwarded to specialized industries that produce chilled, frozen and dried ingredients for the meat and poultry sectors.

According to Annex I of the Commission Regulation (EU) No 142/2011, 'Pet food' means feed for pet animals and dog chews that:

- contain Category 3,
- may contain imported Category 1 material455 comprising of animal by-products derived from animals which have been submitted to illegal treatment as defined in Article 1(2)(d) of Directive 96/22/EC or Article 2(b) of Directive 96/23/EC.

More simply, pet foods are plants or animal materials intended for consumption by pets. Rendering is a process that converts waste animal tissue into value-added materials. Rendering can be carried out on an industrial, farm, or kitchen scale. Of course, pet food industry is an extension of the human food and agriculture industries. Pet food provides a convenient way for slaughterhouse offal, grains considered "unfit for human consumption," and similar waste products to be turned into profit. Catering waste (including catering waste processed in an ABP rendering plant) cannot be used in the manufacture of pet food.

It is known that also the following foods are potentially unsafe for cats and dogs[456]:

- Allium[457]: the toxic principles present in them causes the transformation of hemoglobin into methemoglobin, consequently resulting in hemolytic anemia;
- Raisins and grapes: large doses of raisins can be poisonous to pets and can cause vomiting, diarrhea, lethargy, abdominal pain, lack of appetite and kidney damage;
- Macadamia nuts and walnuts: can cause vomiting, lethargy, hyperthermia, abdominal

and metacarpus bones, tarsus and metatarsus bones, pig bristles, feathers); c) animal by-products from poultry and lagomorphs slaughtered on the farm, which did not show any signs of disease communicable to humans or animals; d) blood; e) animal by-products arising from the production of products intended for human consumption; f) products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons; g) petfood and feedingstuffs of animal origin, or feedingstuffs containing animal by-products or derived products, which are no longer intended for feeding for commercial reasons; h) blood, placenta, wool, feathers, hair, horns, hoof cuts and raw milk; i) aquatic animals, except sea mammals, which did not show any signs of disease; j) material originating from animals which did not show any signs of disease (shells, hatchery by-products, eggs, egg by-products, including egg shells); k) aquatic and terrestrial invertebrates; i) hides and skins, hooves, feathers, wool, horns, hair and fur originating from dead animals that did not show any signs of disease; l) hides and skins, hooves, feathers, wool, horns, hair and fur originating from dead animals that did not show any signs of disease; m) adipose tissue from animals.

455. They are briefly: a) entire bodies and all body parts, including hides and skins of animals suspected of being infected by a TSE, animals other than farmed and wild animals, including in particular pet animals, zoo animals and circus animals, animals used for experiments, animal by-products derived from animals which have been submitted to illegal treatment, containing residues of other substances and environmental contaminants.

456. Gault G., P. Berny, G. Lorgue (1995), "Plants which are toxic for pets", *Recueil de medecine veterinaire*, vol. 171 (2-3): 171-176; Kovalkovičová N., I. Šutiaková, J. Pistl, V. Šutiak (2009), "Some food toxic for pets", *Interdisciplinary Toxicology*, 2 (3): 169-76.

457. Salgado B. S., L. N. Monteiro, N. S. Rocha (2011), "Allium species poisoning in dogs and cats", *Journal of Venomous Animals and Toxins including Tropical Diseases*, vol. 17 (1): 4-11; Cope R. B. (2005), "Allium species poisoning in dogs and cats", *Veterinary Medicine*, 100 (8): 562-6.

pain, stiff joints, lameness and tremors;

- Garlic (in large doses): can cause gastrointestinal problems such as vomiting and diarrhea.
- Onions[458]: damages hemoglobin and is cumulative. They contain a substance (N-propyl disulphide) that destroys red blood cells in the cat;
- Chocolate, coffee-based products[459]: can cause irregular heart rate and rhythm, restlessness, hyperactivity, diarrhea, vomiting, panting, muscle tremors, abdominal pain, bloody urine, increased body temperature, seizures, coma and possibly even death. Theobromine, a component of chocolate, is a toxic compound. Caffeine[460] is also present in chocolate and a toxic component, but in much smaller amounts than Theobromine. Both Theobromine and Caffeine are members of a drug class called Methylxanines;
- Alcoholic beverages: any type of alcohol can be poisonous for pets and aside from intoxication, can cause a coma or even death;
- Apple Seeds: can have varied effects;
- Apricot Pits: can cause respiratory difficulties such as breathing, coughing and sneezing;
- Avocado: can cause digestive problems. It is a very high salicylate that is poisonous to cats and dogs. It will trigger fluid accumulation in the lungs and chest, leading to difficulty breathing and death due to oxygen deprivation. Fluid accumulation can also occur in the heart, pancreas and abdomen;
- Cherry Pits: can cause respiratory difficulties such as breathing, coughing and sneezing.
- Candy containing the sweetener Xylitol: can cause liver damage and even death;
- Mushrooms: contain toxins that will trigger numerous organ systems, including the kidneys, liver and brain. Nervous system abnormalities, seizure, coma, vomiting, and death can all result when a dog ingests mushrooms.

Generally, cooked and marinated foods should be avoided, as well as sauces and gravies, which may contain ingredients that, although well tolerated by humans, may be toxic to animals. Other miscellaneous foods that are toxic to dogs include: raw eggs and egg whites, raw fish, nutmeg, salt, tobacco, trash items, persimmons, yeast and dough containing yeast, liver, marijuana, hops, and human iron supplements.[461]

6. EU rules on animal by-products

One of the first Directives of the European Community was the Council Directive 64/432/EEC.[462] This Directive applied to intra-Community trade in bovine animals and

458. Kobayashi K. (1981), "Onion poisoning in the cat", *Feline Practice*, 11 (1): 22-7; Stallbaumer M. (1981), "Onion poisoning in a dog", Veterinary Record, 108 (24): 523-4; Harvey J. W., D. Rackear (1985), "Experimental onion-induced hemolytic anemia in dogs", *Veterinary Pathology*, 22 (4): 387-92.
459. Eteng M. U., E. U. Eyong, E. O. Akpanyung, M. A. Agiang, C. Y. Aremu (1997), "Recent advances in caffeine and theobromine toxicities: a review", *Plant Foods for Human Nutrition*, 51 (3): 231–243.
460. Tawde S. N., B. Puschner, T. Albin, S. Stump, R. H. Poppenga (2012), "Death by Caffeine: Presumptive Malicious Poisoning of a Dog by Incorporation in Ground Meat", *Journal of Medical Toxicology*, 8 (4): 436-440.
461. Kovalkovičová N., I. Šutiaková, J. Pistl, V. Šutiak (2009), ibid.,169–176.
462. Council Directive of 26 June 1964 "on animal health problems affecting intra-Community trade in bovine animals and swine" (64/432/EEC) (Official Journal L 121, 29.7.1964, p. 164-184). This

swine for breeding, production or slaughter. Each Member State could ensure that only bovine and swine, which fulfill the general conditions, was sent from its territory to that of another Member State. Bovine and swine covered by this Directive could show no clinical sign of disease on the day of loading; had to have been obtained from a holding which was situated in the center of an epizootic free area for at least three months prior to consignment; and had been free from foot-and-mouth disease and bovine brucellosis, swine fever and contagious porcine paralysis (Teschen disease) in the case of swine.

In 1982 the EC Commission, considering the appearance of certain contagious animal diseases, noted a possible risk to the Community herd, as a result of intra-Community trade and in the light of the experience gained from several[463] notifications. Adaptation to technical needs with a procedure calling for close cooperation between the Member States was put into effect and adopted by the Directive 82/894/EEC.[464] Annex I of this Directive reported the diseases which were the subject of the notification: foot-and-mouth disease, rinderpest (cattle plaque), contagious bovine pleuropneumonia, bluetongue, swine vesicular disease, classical swine fever, African swine fever, Teschen disease (contagious swine paralysis), fowl plaque, and Newcastle disease. The ABPs were not yet considered.

In 1985 the first case of Bovine Spongiform Encephalopathy (BSE) was identified in GB. In 1999 the Belgian dioxin[465] crisis was related to chicken feed. Also in 1999 there was the kaolin scandal that involved high level of dioxin added to animal feed in Austria and Germany.[466] Classical swine fever hit in 2000,[467] followed by foot and mouth disease (FMD).[468] These crises, concerning human food and animal feed, exposed weaknesses in

Directive had been many times amended; the last amendments are Directives 97/12 and 98/46.

463. I.e. article 9 of Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade on bovine animals and swine, as last amended by Council of Directive 80/1274/EEC; Article 11 Council Directive 71/118/EEC of 15 February 1971 dealing with health problems affecting trade in fresh poultry meat, as last amended by Council Directive 80/216/EEC; Article 7 of Council Directive 72/461/EEC of 12 December 1972 on health problems affecting intra-Community trade in fresh meat, as last amended by Council Directive 80/1099/EEC and Article 7 of Council Directive 80/215/EEC of 22 January 1980 on animal health problems affecting intra-Community trade in meat products, as last amended by Council Directive 80/1100/EEC.

464. Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community (Official Journal of the European Union, L378 of 31.12.82).

465. Dioxin was cited, in the European press for the first time, in relation to the accident at the chemical plant of Hoffmann-LaRoche in Seveso, Italy. Due to an explosion a great amount of dioxin was spread over the city of Seveso in 1976. In 1999, Belgium had a dioxin crisis caused by dioxin-contaminated feed being fed to livestock. The source of the contamination was a Belgian at-rendering company, where transformer oil with high levels of polychlorinated biphenyls (PCBs) and dioxins was used to manufacture animal foods sale of Belgian poultry and eggs and all food items containing more than 2% egg product.

466. Kaolinite is used, as 3% in animal feed, to improve the flow during pumping when moving the animal feed from one store to another. German kaolin with high level of dioxin had also been added to animal feed in Austria and Germany. In June 1999 animal feedings with added kaolinite were found to have 1,5 to 30 pg i-TE/g resulting in a contamination of German turkeys of 30,6 pg i-TE/g fat.

467. Outbreaks have occurred in 1997-98 in the Netherlands, Germany, Belgium, Spain, Austria, and Switzerland, costing an estimated $ 2.3 billion; 12.4 million animals were slaughtered. In 2000 in the United Kingdom and in 2001 in Germany, Slovakia, Spain, and Romania.

468. Foot and Mouth Disease (FMD) or hoof-and-mouth disease (Aphthae epizooticae) is an infec-

the design and application of food legislation within the EU and lead to the Commission including the promotion of a high level of food safety among its policy priorities. Over the next years, the European Community adopted the Council Directives 90/425/EEC[469] and 90/667/EEC.[470]

According to Council Directive 90/425/EEC, in the Member State of origin, the competent authority must verify, among other things, that the animals and products for trade:

- meet the requirements of the relevant Directives detailed in Annex A (Council Directives 64/432/EEC, 88/407/EEC, 89/556/EEC, 90/426/EEC, 90/429/EEC) and comply with the animal health requirements of the Member State of destination (sheep and goats, live poultry, domestic rabbits, waste, hatching eggs);
- come from centers or organizations which are subject to regular official veterinary checks;
- are accompanied by health certificates and other appropriate documents during transport;
- do not originate from holdings or regions which are subject to restrictions applying to those animals or products because of the suspicion or existence of certain diseases;
- come from a country or region offering sufficient health guarantees from the point of view of the country of destination;
- are transported in accordance with the hygiene rules in force.

Additionally, in the country of origin, a competent authority must carry out checks in holdings, markets and assembly centers to verify that the live animals and products meet European standards, especially as regards identification. Measures would be taken against suppliers or consignors of animals and products who fail to comply with these rules.

Once the transport operation was completed he consignees of animals and products dispatched from another Member State were responsible for those animals and products upon their arrival at the destination and afterwards. If necessary, live animals had to be quarantined at the place of destination or in a quarantine station. Checks had to be carried out at the places where live animals and products from third countries could be brought into EU territory, such as ports, airports and frontier posts with third countries. If there was an outbreak of zoonosis, disease or any other risk to animal or human health, the Member State of dispatch had to take the appropriate preventive and control measures, including restrictions on movement if the risk was serious.

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tious and sometimes fatal viral disease that affects cloven-hoofed animals, including bovids. An outbreak of FMD, Type O, in Uruguay was reported on 27 October 2000. Alert measures in Uruguay had been in place since the August 2000. In the same period FMD outbreaks in Argentina (type A24) and Brazil (type O). An outbreak of FMD in Egypt was reported on 15 September 2000. In 2001, a serious outbreak of FMD in Britain resulted in the slaughter of many animals. Refer for more news: Tully D. C., M. A. Fares (2008), "The tale of a modern animal plague: tracing the evolutionary history and determining the time-scale for foot and mouth disease virus", *Virology*, 382 (2): 250–256.

469. Council Directive 90/425/EEC of 26 June 1990 concerning veterinary and zootechnical checks applicable in intra-Community trade in certain live animals and products with a view to the completion of the internal market (Official Journal of the European Union, No L 224 of 18.8.90).

470. Council Directive 90/667/EEC of 27 November 1990 laying down the veterinary rules for the disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedstuffs of animal or fish origin and amending Directive 90/425/EEC (Official Journal of the European Union, No L 363 of 27.12.90).

According to Article 3 of Council Directive 90/667/EEC, the following high-risk material could be processed in a high-risk processing plant approved by the Member State or disposed of by burning or burial:

- all bovine animals, pigs, goats, sheep, solipeds, poultry and all other animals kept for agricultural production, which have died on the farm but were not slaughtered for human consumption, including stillborn and unborn animals;
- dead animals not referred to in the previous point but which are designated by the competent authority of the Member State;
- animals which are killed in the context of disease control measures either on the farm or in any other place designated by the competent authority;
- animal waste including blood originating from animals which show, during the veterinary inspection carried out at the time of slaughtering, clinical signs of diseases communicable to man or other animals;
- all those parts of an animal slaughtered in the normal way which are not presented for post mortem inspection, with the exception of hides, skins, hooves, feathers, wool, horns, blood and similar products;
- all meat, poultry meat, fish, game and foodstuffs of animal origin which are spoiled and thus present a risk to human and animal health;
- animals, fresh meat, poultry meat, fish, game and meat and milk products, imported from third countries, which in the course of the inspections provided for in Community legislation fail to comply with the veterinary requirements for their importation into the Community, unless they are re-exported or their import is accepted under restrictions laid down in Community provisions;
- without prejudice to instances of emergency slaughtering for reasons of welfare, farm animals which have died in transit;
- animal waste containing residues of substances which may pose a danger to human or animal health; milk, meat or products of animal origin rendered unfit for human consumption by the presence of such residues;
- fish which show clinical signs of diseases communicable to man or to fish.

The competent authorities had where necessary to decide that high-risk material had to be disposed of by burning or by burial where:

- transport to the nearest high-risk material processing plant of animals infected or suspected of being infected with an epizootic disease was rejected because of the danger of propagation of health risks;
- animals are infected with or suspected of being infected with a serious disease or contain residues which could constitute a risk to human or animal health and which could survive inadequate heat treatment;
- a wide-spread epizootic disease lead to a lack of capacity at the high-risk material processing plant;
- animal waste concerned originated from places with difficult access;
- quantity and the distance to be covered did not justify collecting the waste.

Burial had to be deep enough to prevent carnivorous animals from digging up the cadavers or waste and had to be in suitable ground so as to prevent contamination of water tables or any environmental nuisance. Before burial, the cadavers or waste had to be sprinkled as necessary with a suitable disinfectant authorized by the competent authority.

Low-risk material had to be processed in a high-risk or low-risk processing plant approved in accordance with the present law, in a petfood plant, or in a plant preparing pharmaceutical or technical products, or be disposed of by burning or burial in accordance with the present law.

In addition to the animal waste referred to in Article 2 (low-risk material - animal waste other than that covered by Article 3, which does not present serious risks of spreading communicable diseases to animals or man), the following had to be deemed to be low-risk material:

- products excluded under Article 3 (all those parts of an animal slaughtered in the normal way which are not presented for post mortem inspection, with the exception of hides, skins, hooves, feathers, wool, horns, blood and similar products), in so far as they are used in the manufacture of feeding stuffs;
- fish caught in the open sea for the purposes of fishmeal production;
- fresh fish offal from plants manufacturing fish products for human consumption.

The mixture of low-risk material processed together with high-risk material had to be deemed to be high-risk material.

Where low-risk material was processed in a petfood plant or a plant preparing pharmaceutical or technical products, the competent authority may require that it was dispatched, stored and processed in a specific location and under specific conditions.

Fishmeal from industries that received and manufactured exclusively low-risk materials intended for the manufacture of fishmeal had to meet the requirements laid down in Annex II, Chapter III.[471]

The products concerned should be subject to the rules for veterinary checks and any protective measures laid down by Council Directive 90/425/EEC concerning veterinary and zootechnical checks applicable in intra-Community trade of certain live animals and products with a view to the completion of the internal market.

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471. Requirements concerning the products after processing.
1. In the case of high-risk materials, samples of the finished products, taken directly after heat treatment, must be free from heat-resistant pathogenic bacteria spores (Clostridium perfrigens absent in 1 g of the product).
2. Samples of the final products from both low-risk and high-risk material taken during or upon withdrawal from storage at the processing plant must comply with the following standards:
Salmonella: absence in 25 g: n = 5, c = 0, m = 0, M = 0; Enterobacteriaceae: n = 5, c = 2, m = 10, M = 3 × 10$ in 1 g, where:
n = number of units comprising the sample;
m = threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all sample units does not exceed m;
M = maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more sample units is M or more;
C = number of sample units the bacterial count of which may be between m and M, the sample still being considered acceptable if the bacterial count of the other sample units is m or less.

The European directives still do not deal about dangers posed by the use of ABPs, but efforts are by now closer.

In January 2000, the adoption of the "White Paper on Food Safety"[472] followed to these directives, in order to integrate the animal by-products sector into the "farm to table" approach for food safety.

As was stressed at the Helsinki European Council on December 1999, particular attention would be focused on improving quality standards and reinforcing systems of checks throughout the food chain, from farm to table.

The White Paper on food safety was an important element in this strategy. The Commission proposed a number of measures that would enable food safety to be organized in a more coordinated and integrated manner. These included:

- the establishment of an independent European Food Authority with responsibility for independent scientific advice on all aspects relating to food safety, operation of rapid alert systems, and communication of risks;
- an improved legislative framework covering all aspects of food products "from farm to table";
- greater harmonization of national control systems;
- dialogue with consumers and other stakeholders.

The Commission set out the general principles on which European food safety policy should be based:

- a comprehensive, integrated approach throughout the food chain;
- a clear definition of the roles of all stakeholders in the food chain (feed manufacturers, farmers and food operators, the Member States, the Commission, consumers);
- traceability of feed and food and their ingredients;
- a coherent, effective and dynamic food policy;
- risk analysis (comprising risk assessment, management and communication);
- scientific advice to the highest standards of independence, excellence and transparency;
- application of the precautionary principle in risk management.

Since these measures were not considered sufficient, the Commission, based on data provided by each Member State, issued a paper on 20 November 2001.[473] They provided a description of the disposal situation on the disposal and the processing and uses of animal by-products across the Community. The paper covered:

- Processing and disposal routes;
- Trade/export of processed animal protein and rendered fat;

472. Commission of the European Communities, White paper on food safety, COM(1999)719 final, Brussels, 12 January 2000. See for more news: Lauterburg D. (2001), *Food Law: Policy & Ethics*, Cavendish Publishing Ltd., London, pp. 38-49; Spriggs J.,G. Isaac (2001), *Food Safety and International Competitiveness: The Case of Beef*, CABI Publishing, Oxon (UK) – New York (USA), pp. 145-166; Smulders F. J. M., J. D. Collins, Edts. (2002), *Food Safety Assurance and Veterinary Public Health: Food Safety Assurance in pre-harvest phase*, Wageningen Academic Publishers, Nederlands, pp. 17-19.

473. Commission service paper on the processing, disposal and uses of animal by-products in Member States, MEMO/01/378, Brussels, 20 November 2001.

- Collection, transportation and rendering costs;
- Storage capacity for rendered products and costs;
- Incineration, co-incineration and small on-farm incineration;
- Burial and/or landfill;
- Biogas;
- Composting and use of processed animal protein as fertilizer;
- Disposal capacity in Member States.

Finally, in 2002, a Regulation[474] on animal by-products was adopted by the European Parliament and the Council that introduced more stringent conditions throughout the food and feed chains requiring safe collection, transport, storage, handling, processing, uses and disposal of all ABPs.

That Regulation sets up a completely new approach. In the past, raw material of a lower health standard than the one used for human food were permitted for use in animal feeds.

The practice of recycling dead animals and material unfit for human consumption into the feed chain was the main factor in the spreading of the BSE and other food scandals (dioxin crises and foot and mouth disease). The Article 22 of the Regulation (EC) No 1774/2002 prohibited this practice.

The Regulation classified ABPs into three categories based on their potential risk to animals, the public or to the environment and set out how each Category must or may be disposed.

Category 1 (Article 4) materials (i.e. ABPs presented the highest risk. These were "animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed"; or scrapie, "animals other than farmed animals and wild animals, including in particular pet animals, zoo animals and circus animals"; "experimental animals"; "wild animals, when suspected of being infected with diseases communicable to humans or animals" in which there is the presence of residues of prohibited substance e.g. hormone used for growth promotion or environmental contaminants e.g. dioxins, PCBs, "catering waste from means of transport operating internationally") that must be completely disposed of as waste by incineration or landfill after appropriate heat treatment. Category 1 material shall not be imported or exported except in accordance with this Regulation or with rules laid down under the procedure referred to in Article 33 (Where reference is made to this paragraph – "Regulatory procedure", Articles 5 and 7 of Decision 1999/468/EC shall apply).

Category 2 (Article 5) materials. These are other high-risk materials including animal by-products of the following description, or any material containing such by-products: manure and digestive tract content; all animal materials collected when treating waste water from slaughterhouses; products of animal origin containing residues of veterinary drugs;

474. Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption (Official Journal of the European Union, No L 273 of 10.10.2002). Amended and implemented by Commission Regulations (EC) No 79/2005, (EC) No 92/2005, (EC) No 93/2005, (EC) No 2067/2005, (EC) No 181/2006, (EC) No 208/2006, (EC) No 209/2006, (EC) No 1192/2006, (EC) No 2007/2006, (EC) No 1877/2006, (EC) No 185/2007, (EC) No 829/2007, (EC) No 832/2007, (EC) No 1256/2007, (EC) No 1432/2007, (EC) No 1576/2007, (EC) No 399/2008, (EC) No 437/2008, (EC) No 523/2008, (EC) No 777/2008, (EC) No 129/2009).

ABPs presenting a risk of contamination with other animal diseases (e.g. animals which die on farm or are killed in the context of disease control measures on farm) and may be recycled for uses other than feeds after appropriate treatment (e.g. biogas, composting, oleo-chemical products, etc). Category 2 material shall be collected, transported and identified and shall be directly disposed of as waste by incineration in an approved incineration plant or processed in a approved processing plant or transformed in a biogas plant or in a composting plant or disposed of as waste by burial in a landfill, etc.

Category 3 (Article 6) material. These are the lowest-risk materials, that include animal by-products of the following description, or any material containing such by-products: parts of slaughtered animals which are fit for human consumption but are not intended for human consumption for commercial reasons; parts of slaughtered animals which are rejected as unfit for human consumption but are not affected by any signs of diseases communicable to humans or animals; hides and skins, hooves and horns, pig bristles and feathers originating from animals that are slaughtered in a slaughterhouse after undergoing ante-mortem inspection and were fit, as a result of such inspection, for slaughter; blood obtained from animals other than ruminants that are slaughtered in a slaughterhouse and after undergoing ante mortem inspection were found fit as a result of such inspection; former foodstuffs of animal origin, or former foodstuffs containing products of animal origin other than catering waste, which are no longer intended for human consumption for commercial reasons; and shells, hatchery by-products and cracked egg by-products originating from animals which did not show clinical signs of any disease communicable through that product to humans or animals, etc.

The Regulation extended the current ruminant intra-species recycling (cannibalism) ban to other species. Porcine animal by-products could not be fed to pigs and poultry animal by-products could not be fed to poultry. However, derogation was provided for in the case of fish and fur animals subject to strict controls by the competent authority (Article 22).

While the uses of catering waste in feed for pigs and poultry was not the focus of the Regulation, it was of major concern to nearly all Member States. Hence the Member States had agreed on a total ban on such feeding practices in the revised Council Directive on Swine Fever[475].

Such uses of former foodstuffs and restaurant kitchen waste containing meat products were at the origin of a number of major animal disease epidemics, which had led to enormous losses to the farming and non-farming community.

The three EU institutions agreed on the ban on intra-species re-cycling (cannibalism). Because catering waste fed to pigs could contain porcine material, catering waste feeding would be inconsistent with the ban on cannibalism. It was also not possible to establish clear traceability for catering waste. The adopted regulation was flexible, permitting a temporary relaxation of the ban on the use of Category 3 catering waste in feed (Article 19). It requires that only animal by-products derived from animals fit for human consumption (Category 3) could be used for animal feed. In other words, the same health standards required by EU legislation for human food were required for animal feed.

In order to guarantee that ABPs derived from animals unfit for human consumption cannot enter the human food or animal feed chain, the following requirements had been introduced:

475. Council Directive 2001/89/EC of 23 October 2001 on Community measures for the control of classical swine fever.

- complete separation during collection, transport, storage, handling and processing of animal waste not intended for animal feed or human food (Article 7 and 8);
- complete separation of plants dedicated to feed production from plants processing other animal waste destined to destruction (Article 16, etc.);
- stricter rules for traceability of animal by-products, including the control of movements of BSE specified risk material by a record keeping system and accompanying documents or health certificates, and visual markers for animal proteins and fats intended for destruction (Article 7, 8, Chapter III, IV, etc.).

The Regulation introduced a set of controls, which was as strict as the control established for the food industry.

The Commission prepared a series of transitional and permanent implementing measures in order to prepare for and facilitate a swift application of the Regulation on 1 May 2003.[476] The temporary transitional measures covered:

- Feeding catering waste to animals (Austria, Germany);[477]
- Feeding used cooking oil to animals (Ireland, UK);[478]
- To avoid cross-contamination, the Regulation requires total separation between plants handling Category 1, 2 and 3 materials. Transitional measures have been agreed for the separation of oleo-chemical plants (Belgium, Germany, Italy, Netherlands, Spain, Sweden, UK); the separation of processing plants dealing with heat treatment of materials (France, Finland); and the separation of intermediate plants dealing with collection, handling, temporary storage and dispatching (Finland, Italy);
- Low-capacity incinerators/co-incinerators (Finland, UK);[479]
- Manure processing standards (Belgium, France, Finland, Netherlands);
- Composting standards (all Member States)
- Biogas standards (all Member States)
- Processing standards for mammalian blood (Germany, Italy, Spain, UK)
- Collection, transportation and transformation of former foodstuffs (all Member States)

The implementing measures covered:

- A permanent derogation for intra-species recycling of fur animals (Finland and Estonia and Latvia);[480]

476. Jakobsson C., E. B. Sommer, P. De Clercq, G. Bonazzi, J. Schröder (2002), *The policy implementation of nutrient management legislation and effects in some European Countries*, a presentation held on 18th April 2002 in Gent, Belgium at the final Workshop of the EU concerted action Nutrient Management Legislation in European Countries NUMALEC.

477. Germany and Austria had been granted a 4-year transitional period permitting the continued use of swill feeding.

478. Transitional measures for used cooking oil in animal feed allowed industry in the UK and Ireland to adapt their business practices. The transitional measures for used cooking oil lasted on 31 October 2004.

479. Commission Decision 2003/327/EC (Expired on 31/12/2004).

480. 2003/324/EC: Commission Decision of 12 May 2003 as regards a derogation from the intra-species recycling ban for fur animals under Regulation (EC) No 1774/2002 of the European Parliament and of the Council.

- A permanent derogation for the feeding of endangered/protected species of necrophagous (carrion) birds (France, Greece, Italy, Portugal, Spain);[481]
- Rules on the burial and burning of animal by-products (all Member States);[482]
- Rules for low capacity incinerators/co-incinerators (all Member States);[483]
- The introduction of technical amendments to the Annexes (all Member States). Revised models of import health certificates and repealing of old legislation, which apply on 1 May 2004 (Regulation (EC) No. 668/2004). The new health certificates are adapted to an electronic format, allowing for the speedy transfer of trade documents.
- Health conditions have been established for new products such as collagen, egg products, tri-calcium phosphate and flavouring innards.[484]

6.1 Regulation (EC) 1069/2009 and Regulation (EU) 142/2011

In 2009 a new Regulation (EC) No 1069/09[485] passed into European law with the intention of clarifying some of the issues raised by Regulation (EC) No 1774/02 and its derogation and to give a clear framework based on a risk assessment philosophy. Commission Regulation (EU) No 142/11[486] was developed to provide clarity and detailed provisions for the implementation of Regulation (EC) No 1069/09 and implementing Council Directive 97/78/EC as regards certain samples. Testori Coggi[487] reported that the main reasons for changing the regulation were simplification and clarification of rules on environmental issues in conjunction with the parliament's new waste directive. The new ABP Regulation had also to reduce administrative burden on establishments and made science-based modifications to product categorization.

The new EU ABPs Regulation 1069/2009 and Commission Regulation (EU) 142/2011

481. European Commission: Decisions 322/2003 and 830/2005.
482. Commission Regulation (EC) No 811/2003 of 12 May 2003 implementing Regulation (EC) No 1774/2002 of the European Parliament and of the Council as regards the intra-species recycling ban for fish, the burial and burning of animal by-products and certain transitional measures.
483. Commission Regulation (EC) No 808/2003 of 12 May 2003 amending Regulation (EC) No 1774/2002 of the European Parliament.
484. Commission Regulation (EC) No 668/2004 of 10 March 2004 amending certain Annexes to Regulation (EC) No 1774/2002 of the European Parliament and of the Council, as regards the importation from third countries of animal by-products.
485. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (Official Journal of theEuropean Union L 300 of 14.11.2009).
486. Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive (Official Journal of the European Union L 54 of 26.2.2011), amended by Commission Regulation (EU) No 749/2011 of 29 July 2011, Commission Regulation (EU) No 294/2013 of 14 March 2013.
487. Paola Testori Coggi, Deputy director general of DG SANCO, the European Commission's (EC's) Directorate General for Health and Consumer Affairs.

came into force on 4 March 2011 and are referred to as the "EU ABPs Regulation" and the "EU Commission Regulation" respectively.

For most premises, the Regulations require a "covered space to receive animal by-products".[488]

Under EU ABPs Regulation 1069/2009 animal by-products can fall into one of three categories that reflect the level of risk to public and animal health.

According that Regulation "animal by-product" means entire bodies or parts of animals, products of animal origin, and other products obtained from animals, which are not intended for human consumption (article 3). These include: fallen stock on farms, wild animals when they are suspected of being diseased , slaughterhouse waste, skins, feathers, blood, and meat, fish, milk and eggs when they are interned for human consumption (articles 8, 9, 10). More specifically, in other word:

- Category 1 material[489] is defined in article 8 of EU ABPs Regulation 1069/2009. Highest risk and consists principally of material that is considered a TSE risk (Regulation (EC) No 999/2001), and those parts of an animal considered most likely to harbor a disease such as BSE. Pet animals, zoo and circus animals, and experimental animals (article 2(d) of Directive 86/609/EEC) are also classified in this Category due to the level of veterinary drugs and residues they may contain. Wild animals may also be classified as Category 1 material when they are suspected of carrying a disease communicable to humans or animals. Catering waste from means of international transport (i.e. which has come from outside the EU), ABPs collected during the treatment of waste water, and ABPs containing residues of substances and environmental contaminants are listed in Group B(3) of Annex I to Directive 96/23/EC. However if such residues exceed the permitted level laid down by Community legislation, they are also Category 1;
- Category 2 material[490] is defined in article 9 of the same EU ABPs Regulation. Category 2 material is also high risk. It includes fallen stock and manure and digestive tract

488. Annex IX, Section 1, point 1(b) of SANCO/7066/2010 Rev. 4 (POOL/D1/2010/7066/7066R4-EN.doc), Commission Regulation of implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive.

489. According to Article 13 of EU Commission Regulation N° 142/11, the competent authority may authorise the use of Category 1 material consisting of entire bodies or parts of dead animals containing specified risk material for the feeding, in feeding stations, to endangered or protected species of necrophagous birds and other species living in their natural habitat, for the promotion of biodiversity and, outside feeding stations, if appropriate without prior collection of the dead animals, to wild animals, referred to point 1(a) of Section 2 of Chapter II of Annex VI (species of necrophagous birds in the Member States), subject to compliance with the conditions set out in Section 3 of that Chapter (The competent authority must be satisfied, on the basis of an assessment of the specific situation of the species concerned and their habitat, that the conservation status of the species will be improved and must identify in the authorisation, holdings or herds within a geographically defined feeding zone).

490. According to Article 13 of EU Commission Regulation N° 142/11, operators may feed Category 2 and 3 material to the following animals, provided that such material comes from animals which were not killed or did not die as a result of the presence or suspected presence of a disease communicable to humans or animals: zoo animals, fur animals, dogs from recognised kennels or packs of hounds, dogs and cats in shelters, maggots and worms for fishing bait.

content. Category 2 is also the default status of any ABP not defined in the EU ABPs Regulation. These materials include ABPs collected during the treatment of waste water, ABPs from establishments or plants processing Category 2 material, products of animal origin which have been declared unfit for human consumption due to the presence of foreign bodies in those products, products of animal origin other than Category 1 material that are imported or introduced from a third country and fail to comply with Community veterinary legislation for their import or introduction into the Community, fetuses, oocytes, embryos and semen which are not destined for breeding purposes, dead-in-shell poultry.

- Category 3 materials are defined in article 10 of the EU ABPs Regulation. They are considered low risk. Category 3 materials includeparts of animals that have been passed fit for human consumption in a slaughterhouse but which are not intended for consumption. Category 3 also includes products of animal origin, or foodstuffs containing products of animal origin which are no longer intended for human consumption for commercial reasons or due to manufacturing or packaging defects or other defects that do not pose a risk to public or animal health. These may include heads of poultry, hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus and metacarpus bones, tarsus and metatarsus bones of animals other than ruminants requiring TSE testing, pig bristles, feathers, products of animal origin, or foodstuffs containing products of animal origin which are no longer intended for human consumption, pet food and feeding stuffs of animal origin or feeding stuffs containing animal by-products or derived products which are no longer intended for feeding for commercial reasons, blood, placenta, wool, feathers, hair, horns, hoof cuts and raw milk originating from live animals that did not show any signs of disease communicable through that product to humans or animals, shells from shellfish with soft tissue or flesh, hatchery by-products, eggs, egg by-products, including egg shells, day-old chicks killed for commercial reasons, catering waste other than as referred to in article 8.

EU ABPs Regulation requires that mixtures of different categories of animal by-products must assume the categorization of the highest risk animal by-product in the mixture. Therefore a mixture containing categories 1, 2 and 3 would be treated as Category 1 material (articles 8 g and 9 g).

One the most significant change that the EU ABPs Regulation introduced is the concept of end point in the manufacturing of ABPs, beyond which the processed products are no longer subject to the requirements of the EU ABPs Regulation due to the eliminated potential risks via heat or chemical substances (Article 5). Consequently, certain finished products are excluded from the scope of Regulation 1069/2009 to reduce operational burdens such as labeling or record-keeping. This simplification provided v for the management of small quantities.[491]

491. The point 52) of "Whereas": "Certain establishments or plants which handle only small quantities of animal by-products which do not pose a risk to public and animal health should be allowed to dispose of such by-products by means other than disposal in accordance with this Regulation, under official supervision". According to point 55): "For materials typically sent in small quantities for research, educational, artistic or diagnostic use, special conditions should be laid down to facilitate the movement of such materials within the Community. Bilateral arrangements facilitating the control of

According to Article 3 of EU Commission Regulation No 142/2011 the following derived products may be placed on the market, and imported, without restrictions:

a. biodiesel which fulfills the requirements for the disposal and use of derived products set out in point 2(b) of Section 3 of Chapter IV of Annex IV;[492]
b. processed petfood which fulfills the specific requirements for processed petfood set out in point 7(a) of Chapter II of Annex XIII;[493]
c. dogchews which fulfill the specific requirements for dogchews set out in point 7(b) of Chapter II of Annex XIII;[494]
d. hides and skins of ungulates which fulfill the specific requirements for the end point of those products set out in point C of Chapter V of Annex XIII;[495]
e. wool and hair which fulfill the specific requirements for the end point for those products set out in point B of Chapter VII of Annex XIII;[496]
f. feathers and down which fulfill the specific requirements for the end point for those products set out in point C of Chapter VII of Annex XIII;[497]
g. fur which fulfills the special requirements for the end point for that product set out in Chapter VIII of Annex XIII;[498]
h. fish oil for the production of medicinal products which fulfills the special requirements for the end point for that product set out in Chapter XIII of Annex XIII;[499]

materials moved between the Member States sharing a common border should be permitted under special circumstances".

492. Materials resulting from processing in accordance with the biodiesel production process may be in the case of biodiesel and of residues from the distillation of biodiesel, used as a fuel without restrictions under this Regulation (end point) and used for the production of derived products for application to land, in the case of potassium sulphate.

493. Processed petfood which has been manufactured and packaged in the Union in accordance with point 3 and which has been tested in accordance with point 5 or which has been subject to veterinary checks in accordance with Directive 97/78/EC at a border inspection post, may be placed on the market without restrictions.

494. Dogchews which have been manufactured and packaged in the Union in accordance with point 4 and which has been tested in accordance with point 5 or which have been subject to veterinary checks in accordance with Directive 97/78/EC at a border inspection post, may be placed on the market without restrictions.

495. Hides and skins of ungulates which pursuant to the decision of an operator are destined for purposes other than human consumption, and which comply with the requirements of Regulation (EC) No 853/2004 for raw materials for gelatine or collagen intended for use in food may be placed on the market without restrictions. Hides and skins having undergone the complete process of tanning, 'wet blue', 'pickled pelts', limed hides (treated with lime and in brine at a pH of 12 to 13 for at least eight hours) may be placed on the market without restrictions.

496. Factory-washed wool and hair, and wool and hair which has been treated by another method which ensures that no unacceptable risks remain, may be placed on the market without restrictions.

497. Feathers, parts of feathers and down which have been factory-washed and treated with hot steam at 100 °C for at least 30 minutes may be placed on the market without restrictions.

498. Furs which have been dried at an ambient temperature of 18 °C for two days at a humidity of 55% may be placed on the market without restrictions.

499. Fish oil derived from the materials referred to in point A.2 of Section 3 of Chapter II of Annex X, which has been de-acidified with a NaOH solution at a temperature of 80 °C or more and which has

i. gasoline and fuels that fulfill the specific requirements for products from the multi-step catalytic process for the production of renewable fuels set out in point 2(c) of Section 3 of Chapter IV of Annex IV.[500]

The following uses of ABPs and derived products are prohibited:[501]

- feeding of terrestrial animals of a given species other than fur animals with processed animal protein derived from the bodies or parts of bodies of animals of the same species;
- feeding of farmed animals other than fur animals with catering waste or feed material containing or derived from catering waste;
- feeding of farmed animals with herbage, either directly by grazing or by feeding with cut herbage, from land to which organic fertilizers or soil improvers, other than manure, have been applied unless the cutting or grazing takes place after the expiration (and after at least 21 days) of a waiting period which ensures adequate control of risks to public and animal health;
- feeding of farmed fish with processed animal protein derived from the bodies or parts of bodies of farmed fish of the same species (Article 11).

The available system for the disposal and use of animal by-products vary with the Category and are listed in articles 12 (Category 1), 13 (Category 2) and 14 (Category 3) of the EU ABPs Regulation. In general the higher the risk Category the fewer are the options for use.

The detailed rules on use and disposal are found in the EU Commission Regulation No 142/11.

Article 16 of the EU ABPs Regulation permits member States to avail themselves of certain derogations for the use and disposal of animal by-products. By way of derogation from articles 12, 13, and 14, animal by-products may be used for research and other specific purposes in accordance with article 17 (research and other specific purposes) in the case of animal by-products referred to in article 18 (special feeding purposes) that are used for special feeding purposes, such as in the case of animal by-products referred to in article 19 (collection, transport and disposal); and are disposed of in accordance with that article (a competent authority may authorize the disposal by burial of dead pet animals and equidae by burning or burial on site or by other means under official supervision which prevent the transmission of risks to public); and are disposed of or used in accordance with alternative methods which have been authorized in accordance with article 20 (authorization of alternative methods) in the case of Category 2 and Category 3 materials; and if authorized by the competent authority and used for the preparation and application to land of bio-dynamic

subsequently been purified by distillation at a temperature of 200 °C or more, may be placed on the market for the production of medicinal products without restrictions.

500. The multi-step catalytic process for the production of renewable fuels may be in the case of gasoline and the other fuels resulting from the process, used as a fuel without restrictions. The multi-step catalytic process for the production of renewable fuels may be, in the case of used clay from bleaching and sludge from the pre-treatment process, disposed of by incineration or co-incineration, transformed into biogas, composted or used for the manufacture of derived products referred to in Article 36(a)(i) of Regulation (EC) No 1069/2009.

501. The document, from the European Parliament's Committee on the Environment, Public Health and Food Safety, stops short of altering the status quo on cattle feed, pointing out that because cows are vegetarian they should not be fed so-called "processed animal proteins" (PAP). The same goes for sheep.

preparations as referred to in article 12-1c (the use of biodynamic preparations is allowed) of Regulation (EC) No 834/2007;[502] and in the case of Category 3 material; and if authorized by the competent authority are used for feeding to pet animals, in the case of ABPs, except for Category 1 material, which arise in the course of surgical intervention on live animals or during birth of animals on farm and, if authorized by the competent authority, disposed of on that farm.

Article 20 of the EU ABPs Regulation provides for the authorization of alternative methods for use and disposal of ABPs. The procedure for authorization of an alternative method of use or disposal of ABPs or derived products may be initiated either by the Commission or, following an application, by a Member State or by an interested party that may represent several interested parties.

The guidance in the Section 1 of the EU ABPs Regulation (Title II – Obligations of operators) explains the registration, transportation, documentation and record keeping requirements for premises handling ABPs. Additionally the guidance explains under what circumstances bacteriological sampling is required and how this should be done.

The procedures for loading and unloading animal by-products at EU ABPs Regulation approved premises must be agreed upon with the inspecting officer prior to issue of the approval or registration, and must be included in the plant's "standard operating procedure" or HACCP plan. They must be designed to ensure that ABPs are unloaded/loaded with minimal risk to animal or public health. In general, ABPs should be unloaded/loaded inside the building. With the agreement of an inspecting officer and a permit, certain ABPs could be unloaded in outside yards in tightly controlled circumstances, providing the ABPs are moved into the building without delay. If unloading outside is permitted, the plant's construction and operations must meet the agreement of the inspecting officer and must ensure the following:

- the premises must have an impervious yard, constructed in a manner that allows it to be cleaned and disinfected, and with a fall to a foul drain;
- the vehicle must park on the impervious yard and unloading must be carried out as quickly as possible;
- the receiving container is leak proof and is securely covered immediately after loading;
- the receiving container is stored on an impervious surface constructed in a manner that allows it to be cleaned and disinfected, and with a fall to a foul drain;
- immediate clean-up procedures must be in place in case of spillage.

Before commencing operations, operators are required to notify the competent authority of establishments or plants under their control which are active at any stage of the generation, transportation, handling, processing, storage, placing on the market, distribution, use or disposal of ABPs and derived products (Article 23 - EU ABPs Regulation) in order to be either approved or registered before commencing operations. This requirement is subject to some exceptions. Article 24 (EU ABPs Regulation) lists the type of establishments/activities that require approval before they can operate:

502. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91 (Official Journal of the European Union, L 189 of 20.7.2007).

a. Incinerators (high and low capacity);
b. Compost (except home composting), biogas/anaerobic digestion;
c. Processing plants;
d. Biodiesel plants;
e. Petfood plants: hermetically sealed, chews, heated, raw, including petfood manufactured in domestic kitchens;
f. Handling plants: all plants that carry out sorting, cutting, chilling, freezing, salting or other preservation process, removal of hides and skins, removal, hygienization or pasteurization, sieving, etc.;
g. Storage plants: all plants that store raw material and send it out again in the same state;
h. Storage of derived products, plants that take in certain derived products (those intended to be disposed of in landfill or by incineration or to be used as fuel for combustion, used as feed (excluding plants approved under Regulation (EC) No 183/2005[503]), use for organic fertilizers/soil improvers);
i. Boilers using tallow as fuel;
j. Organic fertilizer/soil improver manufacturers.

The following activities require registrations unless they are approved as above:

a. Manufacturers using wool, hair, pig bristles, feathers, apiculture by-products, bone, bone products, horns, horn products, hooves, hoof products, milk, milk-based products, milk-derived products, colostrum, colostrum-based products;
b. Manufacturers of blood, blood products, hides and skins and products thereof, tanning activities, commercial game trophies, oleochemicals;
c. Collection centers;
d. Users of ABPs to feed zoo/circus animals or other wild animals or special feeding uses;
e. Research and diagnostic samples;
f. Mixers of components to manufacture organic fertilizers/soil improvers;
g. Manufacturers of cosmetic products, active implantable medical devices, medical devices, in vitro diagnostic medical devices, veterinary medicinal products, medicinal products, handlers of intermediate products;
h. Other operators as landfill sites, pet cemeteries, operators who place untreated wool or hair (from farms) on the market;
i. Operators who receive hides and skins from their own animals back from abattoirs;
j. Operators who handle material as trade samples or for exhibition or artistic uses.
k. Haulers and transporters.

503. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (Official Journal of the European Union L 35 of 8.2.2005).

Activities that do not require ABP registration:

- Plants that are approved or registered in accordance with Regulation (EC) No. 852/2004 or Regulation (EC) No. 853/2004;
- Plants listed above as requiring approval;
- Activities where ABPs are generated by farms and other places where animals are kept, bred or taken care of.

According article 6 of EU Commission Regulation No 142/2011, the competent authority has to ensure that incineration and co-incineration of animal by-products and derived products will only take place in incineration plants and co-incineration plants which have been granted a permit in accordance with Directive 2000/76/EC or for plants not required to have a permit under the same Directive, in incineration and co-incineration plants which have been approved by the competent authority to carry out disposal by incineration, or disposal or recovery of animal by-products or derived products, if they are waste, by co-incineration. Operators of incineration plants and co-incineration plants shall comply with the general requirements for incineration and co-incineration set out in Chapter I of Annex III.[504] Operators of high-capacity and low-capacity incineration and co-incineration plants shall comply with the requirements of Chapter II[505] and Chapter III of Annex III.[506]

In derogation from article 12 (Disposal and use of Category 1 material) and Article 14(c) (disposed of in an authorized landfill, following processing) of EU ABPs Regulation No 1069/2009, the competent authority may authorize the disposal of the following Category 1 and 3 materials in an authorized landfill:

a. imported petfood or petfood produced from imported materials, from Category 1 material referred to in article 8(c) (animal by-products derived from animals which have been submitted to illegal treatment) of EU ABPs Regulation No 1069/2009;

504. Among the main requirements we report the following. Operators of incineration and co-incineration plants shall ensure that the hygiene conditions (ABPs and derived products must be disposed of as soon as possible after arrival, animals must not have access to the plants, ABPs and derived products that are awaiting incineration or co-incineration or to ash resulting from their incineration, there must be total physical separation between the incineration or co-incineration equipment and the livestock, equipment must be dedicated entirely to the operation of the incinerator and not used elsewhere on the holding, incompletely incinerated animal by-products must be reincinerated or disposed of by other means) are met in the plants under their control.

505. Incineration or co-incineration plants treating only ABPs and derived products with a capacity of more than 50 kg per hour (high-capacity plants) and which are not required to have a permit to operate in accordance with Directive 2000/76/EC shall comply with the following conditions: The plants must be equipped for each line with at least one auxiliary burner that will be switched on automatically when the temperature of the combustion gases after the last injection of combustion air falls below 850 °C or 1100 °C, as applicable. When ABPs or derived products are introduced into the incineration chamber, the plant must operate an automatic system to prevent the introduction of ABPs until the temperature of 850 °C or of 1100 °C has been reached.

506. Incineration and co-incineration plants treating only ABPs and derived products with a maximum capacity of less than 50 kg of ABPs per hour or per batch (low-capacity plants) and which are not required to have a permit to operate in accordance with Directive 2000/76/EC shall only be used for the disposal of dead pet animals or Category 1 materials referred to in article 8(b), (e) and (f), Category 2 materials referred to in article 9 or Category 3 materials referred to in article 10 of Regulation (EC) No 1069/2009.

b. Category 3 material referred to in article 10(f) (products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial) and (g) (petfood and feedingstuffs of animal origin, which are no longer intended for feeding for commercial reasons) of EU ABPs Regulation No 1069/2009 (Article 7 of Commission Regulation (EU) No 142/2011).

According of article 10 of EU Commission Regulation No 142/2011, operators shall ensure that establishments and plants under their control comply with the following requirements for the transformation of ABPs and derived products into biogas or for composting:

- the requirements applicable to biogas and composting plants set out in Chapter I (requirements applicable to plants);
- the hygiene requirements applicable to biogas and composting plants set out in Chapter II (hygiene requirements applicable to biogas and composting plants);
- the standard transformation parameters set out in Section 1 of Chapter III (Standard transformation parameters);
- the standards for digestion residues and compost set out in Section 3 of Chapter III (standards for digestion residues and compost).

The competent authority will only approve biogas and composting plants if they comply with the requirements laid down in Annex V of EU Commission Regulation No 142/2011. A biogas plant must be equipped with a pasteurization/hygienisation unit, which cannot be by-passed for the animal by-products or derived products introduced with a maximum particle size of 12 mm before entering the unit. The plant must also have equipment for monitoring that the temperature of 70 °C is reached within one hour and has recording devices and a system to prevent insufficient heating. A composting plant must be equipped with a closed composting reactor or closed area which cannot be by-passed for the ABPs or derived products introduced into the plant, and it must be equipped with installations for monitoring temperature against time, recording devices to record the results of the monitoring measurements of temperature, and an adequate safety system to prevent insufficient heating.

When a plant receives or dispatches animal by-products, ideally the vehicle will enter the building for unloading or loading. This seems the only acceptable method for many by-products such as slaughter and butchery waste, or where by-products are tipped onto the floor or into a hopper.

According to article 20 of EU Commission Regulation (Requirements concerning certain registered establishments and plants handling animal by-products and derived products):

1. Operators of registered plants or establishments or other registered operators shall handle ABPs and derived products under the conditions set out in Chapter IV[507]

507. 1. Operators of registered plants or establishments or other registered operators shall handle animal by-products and derived products under the following conditions:
- premises must be constructed in a way permitting their effective cleaning and disinfection, where appropriate;
- premises must have appropriate arrangements for protection against pests, such as insects, rodents and birds;
- installations and equipment must be kept in hygienic condition, where necessary;
- animal by-products and derived products must be stored under conditions preventing contamination.
Operators shall keep records in a form which is accessible to the competent authority.

of Annex IX (Requirements applicable to certain approved and registered establishments and plants).

2. Registered operators transporting ABPs or derived products, other than between premises of the same operator, shall in particular comply with the conditions set out in point 2 of Chapter IV of Annex IX.

   Paragraphs 1 and 2 shall not apply to:

   a. approved operators who are transporting animal by-products or derived products as an ancillary activity;

   b. operators who have been registered for transport activities in accordance with Regulation (EC) No 183/2005.

Hides and skins are also treated as ABPs unless they are being used for the production of gelatin and/or collagen for human consumption,[508] in which case they must have come from animals that have passed ante and post mortem inspection and their storage must comply with the requirements for fresh meat in the food hygiene legislation. They must also be kept separate from hides and skins categorized as ABPs (Article 26 - EU ABPs Regulation). The handling of hides and skins can be considered in a number of different circumstances:

- at abattoirs where animals under or over 48 months of age are slaughtered;
- at animal by-product plants that recover fallen stock hides where the fallen stock are under or over 48 months of age;
- at stand-alone approved animal by-product hide stores or at collection centers and tanneries.

Control of hides and skins derived from cattle over 48 months of age slaughtered for human consumption and fallen cattle are subject to BSE testing. Hides of animals over 48 months of age can be recovered for use in the leather industry provided they have come from animals that have been tested for Transmissible Spongiform Encephalopathy (TSE) with a negative result. This includes hides from fallen stock over 48 months of age as well as those slaughtered for human consumption.

Operators have to put in place, implement and maintain own checks in their establishments or plants in order to monitor compliance with this Regulation. They have to ensure that no animal by-products or derived products suspected or discovered that do not to comply with this Regulation leave the establishment or plant, unless destined for disposal (Article 28 - EU ABPs Regulation).

Handling and storage plants require approval under the EU ABPs Regulation. They must be adequately separated from other plants such as slaughterhouse and food handling

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Registered operators transporting animal by-products or derived products, other than between premises of the same operator, shall in particular:
- have information at their disposal with regard to the identification of their vehicles, which allows the verification of the use of the vehicles for the transport of animal by-products or derived products;
- clean and disinfect their vehicles, as appropriate;
- take all other necessary measures to prevent contamination and the spreading of diseases communicable to humans or animals.

508. Paragraph 5, Chapter I, Section XV, Annex III of Regulation (EC) 853/2004 regarding the requirements for collection centres and tanneries supplying hides and skins for the manufacture of gelatine for human consumption (the same requirements apply for collagen).

premises. They may receive ABPs from other premises, and all consignments of ABPs must be accompanied by a commercial document.

ABPs and derived products destined for feeding to farmed animals, excluding fur animals, may only be placed on the market provided they are or they are derived from Category 3 material other than material referred to in article 10 (hides and skins, hooves, feathers, wool, horns, hair and fur, (adipose tissue, and catering waste) (Article 31 - EU ABPs Regulation).

Organic fertilizers and soil improvers may be placed on the market and used provided they are derived from Category 2 or Category 3 material. In addition, digestion residues from transformation into biogas or compost may be placed on the market and used as organic fertilisers or soil improvers (Article 32 - EU ABPs Regulation). Article 22 of EU Commission Regulation states that the competent authority of the Member State where an organic fertilizer or a soil improver, which has been produced from meat-and-bone meal derived from Category 2 material or from processed animal protein, is to be applied to land shall authorize one or more components which are to be mixed with those materials.

Cosmetic products, active implantable medical devices, in vitro diagnostic medical devices, and veterinary medicinal products, may be placed on the market without restrictions (Article 33 - EU ABPs Regulation). Pet food derived from Category 3 material may be placed on the market (Article 35 - EU ABPs Regulation).

Operators may place on the market derived products, other than the products referred to in articles 31 (ABPs and derived products destined for feeding to farmed animals, excluding fur animals), 32 (Organic fertilizers and soil improvers, digestion residues from transformation into biogas or compost), 33 (cosmetic, active implantable medical devices, medical devices, in vitro diagnostic medical devices, veterinary medicinal products) and 35 (petfood), provided those products are not intended for use for the feeding to farmed animals or for application to land from which such animals are to be fed or intended for feeding to fur animals. Operators ensure the control of risks to public and animal health by safe sourcing in accordance with article 37, safe treatment in accordance with article 38, and verifying that the products are only used for safe end uses in accordance with article 39 where safe treatment does not ensure sufficient control (Article 36 of EU ABPs Regulation). Article 24 of the EU Commission Regulation confirms that the use of Category 1 material for the manufacture of derived products that are intended to be ingested by or applied to humans or animals, other than for derived products referred to in Articles 33 and 36 of EU ABPs Regulation, shall be prohibited. Article 25 of the EU Commission Regulation states that the importation into and the transit through the Union of the following ABPs shall be prohibited: unprocessed manure, untreated feathers and parts of feathers and down, beeswax in the form of honeycomb. Wool and hair which has been factory-washed and furs which have been dried at an ambient temperature of 18°C for a period of at least two days at a humidity of 55% can be imported or may transit through the Union without any animal health conditions.

As a consequence, factory-washed wool and wool which has been treated by another method which ensures that no unacceptable risks remain, may be placed on the market without restrictions. Only processed wool could be placed on the market; unprocessed wool could only be sent to intermediate plants for storage and/or technical plants for sorting and/or washing. “Unprocessed wool” or “untreated wool” is defined in the new EU Commission Regulation as: “wool that has not undergone factory washing, been obtained from tanning, or been treated by some other method that ensures that no unacceptable risks remain”.

The export of ABPs and derived products destined for incineration or landfill is prohibited. The export of ABPs and derived products to third countries that are not members of the OECD for use in a biogas or composting plant is prohibited (Article 43 - EU ABPs Regulation).

Each Member State will draw up a list of establishments, plants and operators that have been approved or registered in accordance with this Regulation within its territory (Article 47 - EU ABPs Regulation).

7. EU rules on waste from ABPs

Already mentioned at the beginning: that the unused ABPs have to be classified as waste and then destined for disposal. Most European waste policy and guidance is based on EU legislation that gives strong direction on waste issues to member states. The main instruments of establishing law and policy are the Directives that specify the objectives that the EU seeks to achieve on particular issues of waste management. It is necessary for member states to implement their requirements within prescribed timescales. Let us recall the main waste directive.

7.1 Waste Directive

Waste Framework Directive 75/442/EEC [509]

This was the original framework directive on waste which was amended by 91/156/EEC, 91/92/EEC, and the Commission Decision 96/350/EC. The directive relates to waste disposal and the protection of the environment from harmful effects caused by the disposal of waste. In particular it aims to encourage the recovery and use of waste in order to conserve natural resources.

Wastes are defined (Article 1) as "any substance or object in the categories set out in Annex I (Q1 – Production or consumption residues not otherwise specified below; Q2 – Off-specification products; Q3 – Products whose date for appropriate use has expired; Q4…etc…; Q16 – Any materials, substances or products which are not contained in the above categories) which the holder discards or intends or is required to discard". The scope of this directive excludes certain categories of waste (Article 2: gaseous effluents emitted into the atmosphere; radioactive waste; waste resulting from prospecting, extraction, treatment and storage of mineral resources and the working of quarries; animal carcasses and the following agricultural waste: fecal matter and other natural, non dangerous substances used in farming; waste water, with the exception of waste in liquid form; decommissioned explosives).

The directive required member states to appoint competent authorities to draw up waste management plans (Article 7) and to develop an integrated network of regional facilities (Article 5). The directive also established the requirements for licenses, registration of carriers (Article 9), and the polluter pays principle (Article 15).

Animal carcasses and ABPs are excluded from the aim of this directive.

509. Council Directive 75/442/EEC of 15/7/1975 on waste (Official Journal of the European Communities L 194 of 25.7.1975).

Waste Framework Directive 2008/98/EC [510]

This Directive repealed Directive 2006/12/EC of the European Parliament and of the Council of 5 April 2006 regarding waste (the codified version of Directive 75/442/EEC as amended), hazardous waste Directive 91/689/EEC, and the Waste Oils Directive 75/439/EEC. It provides for a general framework of waste management requirements and sets the basic waste management definitions for the EU.

In its premise this Directive states that "The first objective of any waste policy should be to minimize the negative effects of the generation and management of waste on human health and the environment. Waste policy should also aim at reducing the use of resources and favor the practical application of the waste hierarchy. In its Resolution of 24 February 1997[511] on a Community strategy for waste management, the Council confirmed that waste prevention should be the first priority of waste management, and that re-use and material recycling should be preferred to energy recovery from waste, where and insofar as they are the best ecological options. It is therefore necessary to revise Directive 2006/12/EC[512] in order to clarify key concepts such as the definitions of waste, recovery and disposal, to strengthen the measures that must be taken in regard to waste prevention, to introduce an approach that takes into account the whole life-cycle of products and materials and not only the waste phase, and to focus on reducing the environmental impacts of waste generation and waste management, thereby strengthening the economic value of waste. Furthermore, the recovery of waste and the use of recovered materials should be encouraged in order to conserve natural resources".

According to Article 2, from the scope of this Directive, to the extent that they are covered by other Community legislation, are excluded:

a. ABPs including processed products covered by Regulation (EC) No 1774/2002, except those which are destined for incineration, landfilling or use in a biogas or composting plant;
b. carcasses of animals that have died other than by being slaughtered, including animals killed to eradicate epizootic diseases and that are disposed of in accordance with Regulation (EC) No 1774/2002.

The definition of "waste"[513] is much simpler than that of the Directive 75/442/EEC. Within the scope of this Directive fall the parts above mentioned of ABPs which are destined for incineration, landfilling or use in a biogas or composting plant. According to Article 4, the following waste hierarchy will apply as a priority order in waste prevention and management legislation and policy:

a. prevention;
b. preparing for re-use;
c. recycling;
d. other recovery, e.g. energy recovery;
e. disposal.

510. European Parliament and Council Directive 2008/98/EC of 19 November 2008 on waste and repealing certain directives (Official Journal of the European Communities L 312/3 of 22 November 2008).
511. Official Journal of the European Communities C 76, 11.3.1997.
512. Directive 2006/12/EC had amended Directive 75/442/EEC.
513. Article 3: 'waste' means any substance or object which the holder discards or intends or is required to discard.

For ABPs the recycling, energy recovery, and disposal are applicable. The recovered parts may be regarded as being by-products because the conditions of articles 5 and 6 are met.

According to article 7, the measures designed to amend non-essential elements of this Directive relating to the updating of the list of waste (known as CER codes) established by Decision 2000/532/EC[514] will be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 39 (2).[515] The list of waste will include hazardous waste and will take into account the origin and composition of the waste and, where necessary, the limit values of concentration of hazardous substances. The list of waste will be binding as regards determination of the waste which is to be considered as hazardous waste.

In the list of wastes, ABPs can be classified with the following codes:

0.2 Wastes from agricultural, horticultural, hunting, fishing and aquacultural primary production, food preparation and processing;

02 01 02 - Animal tissue waste (primary production wastes) ;

02 02 02 - Animal tissue waste (wastes from the preparation and processing of meat, fish and other foods of animal origin);

02 02 03 - Material unsuitable for consumption or processing (wastes from the preparation and processing of meat, fish and other foods of animal origin).

As can be seen, ABPs are not considered dangerous. In reality, in the case of ABPs classified in Category 1 and 2 materials, the same CERs equipped with asterisk[516] can be used, or the following:

02 01 99* (Animal tissue waste);

02 02 99* (Animal tissue waste).

As in Article 9, in order to strengthen the re-use and the prevention, recycling and other modes of waste recovery (and so ABPs), Member States may take legislative or non-legislative measures to ensure that any natural or legal person who professionally develops, manufactures, processes, treats, sells or imports products (producer of the product) has extended producer responsibility.

As in Article 22 (Bio-waste),[517] Member States are encouraged will take measures, as appropriate, and in accordance with Articles 4 and 13, and to encourage:

514. Commission Decision 2000/532/EC of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste (Official Journal of the European Communities L 226 of 6.9.2000).

515. Where reference is made to this paragraph, Article 5(1) to (4) and Article 7 of Decision 1999/468/EC shall apply, having regard to the provisions of Article 8 thereof. (Article 5.1: The Commission shall be assisted by a regulatory committee composed of the representatives of the Member States and chaired by the representative of the Commission).

516. According to the Annex of the Decision 2000/532/EC, "Any waste marked with an asterisk (*) is considered as a hazardous waste pursuant to Article 1(4), first indent, of Directive 91/689/EEC on hazardous waste".

517. "Bio-waste" (Article 3) means biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants. The ABPs may be included among the bio-wastes.

a. the separate collection of bio-waste with a view to composting and digestion of bio-waste;

b. the treatment of bio-waste in a way that fulfills a high level of environmental protection;

c. the use of environmentally safe materials produced from bio-waste.

Member States will require any establishment or undertaking intending to carry out waste treatment to obtain a permit from the competent authority (Article 23).

As in Article 26, where the following are not subject to permit requirements, Member States will ensure that the competent authority keeps a register of:

a. establishments or undertakings which collect or transport waste on a professional basis;

b. dealers or brokers;

c. establishments or undertakings which are subject to exemptions from the permit requirements pursuant to Article 24 (exemptions from permit requirements).

Annex I lists the disposal operations: 1. Deposit into or on to land (e.g. landfill, etc.); 2. Land treatment (e.g. biodegradation of liquid or sludgy discards in soils, etc.); 3. Deep injection (e.g. injection of pumpable discards into wells, salt domes, or naturally occurring repositories, etc.); 4. Surface impoundment (e.g. placement of liquid or sludgy discards into pits, ponds or lagoons, etc.); 5. Specially engineered landfill (e.g. placement into lined discrete cells which are capped and isolated from one another and the environment, etc.); 6. Release into a water body except seas/oceans; 7. Release to seas/oceans including sea-bed insertion; 8. Biological treatment not specified elsewhere in this Annex which results in final compounds or mixtures which are discarded by means of any of the operations numbered 1 to 12; 9. Physico-chemical treatment not specified elsewhere in this Annex which results in final compounds or mixtures which are discarded by means of any of the operations numbered 1 to 12 (e.g. evaporation, drying, calcination, etc.); 10. Incineration on land; 11. Incineration at sea (This operation is prohibited by EU legislation and international conventions); 12. Permanent storage (e.g. emplacement of containers in a mine, etc.); 13. Blending or mixing prior to submission to any of the operations numbered 1 to 12; 14. Repackaging prior to submission to any of the operations numbered 1 to 13; 15. Storage pending any of the operations numbered 1 to 14 (excluding temporary storage, pending collection, on the site where the waste is produced).

7.2 Landfill Directive

The target of the Directive 99/31/EC[518] was to provide for measures, procedures and guidance to prevent or reduce, as far a possible, the negative environment effects of landfilling waste. It also aimed to reduce methane emissions and to harmonized the controls on landfill throughout the European Union by setting targets for a reduction in the volumes of biodegradable municipal waste going to landfills.

It defined the different categories of waste (municipal waste, hazardous waste, non-hazardous waste and inert waste) and applied that definition to all landfills designed as waste disposal sites for the deposit of waste onto or into land. Landfills were divided into three classes (Article 4):

518. Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste (Official Journal of the European Communities L 182 of 16/07/1999).

- landfills for hazardous waste (any waste which was covered by Article 1(4) of Council Directive 91/689/EEC of 12 December 1991 on hazardous waste);
- landfills for non-hazardous waste (any substance or object which was covered by Directive 75/442/EEC);
- landfills for inert waste (waste that did not undergo any significant physical, chemical or biological transformations. Inert waste does not dissolve, burn or physically or chemically react, biodegrade or adversely affect other matter with which it comes into contact in a way likely to give rise to environmental pollution or harm human health. The total leachability and pollutant content of the waste and the ecotoxicity of the leachate was insignificant and did not endangered the quality of surface water and/or groundwater).

The Directive (Article 3, p. 2) did not apply to:

- the spreading of sludges on the soil (including sewage sludge and sludge resulting from dredging operation and similar matter for the purpose of fertilization or improvement);
- the use of inert waste in landfills for redevelopment or restoration work, or for construction purposes;
- the deposit of unpolluted soil or of non-hazardous inert waste resulting from prospecting and extraction, treatment and storage of mineral resources and from the operation of quarries;
- the deposit of non-hazardous dredge sludges alongside small waterways from which they have been dredged and of non-hazardous sludges in surface water, including the bed and its subsoil.

A standard waste acceptance procedure (Article 6) is laid down so as to avoid any risks:

- waste must be treated before being landfilled;
- hazardous waste within the meaning of the Directive must be assigned to a hazardous waste landfill;
- landfills for non-hazardous waste must be used for municipal waste and for non-hazardous waste;
- landfill sites for inert waste must be used only for inert waste;
- criteria for the acceptance of waste at each landfill class must be adopted by the Commission in accordance with the general principles of Annex II (Waste acceptance criteria and procedures).

The following wastes (Article 5, p. 3) are not to be accepted in a landfill:

a. liquid waste;
b. waste which, in the conditions of landfill, is explosive, corrosive, oxidizing, highly flammable or flammable as defined in Annex III to Directive 91/689/EEC;
c. hospital and other clinical waste which is infectious;
d. used tires, with certain exceptions;
e. any other type of waste which does not meet the acceptance criteria laid down in Annex II.

In terms of environmental protection the directive contains detailed provisions for the permit (articles 8 and 9), cost (Article 10), acceptance procedures (Article 11), control and monitoring procedures (Article 12), closure, and after-care procedures (Article 13). The requirements will apply immediately to all new landfills, and existing sites will have to be brought up to the higher standards or will have to close. All current licenses will need to be reviewed and there will be a transitional period, extending up to 2009, for bringing existing sites up to the required standard..

7.3 Packaging and Packaging Waste Directive

The EU first introduced measures on packaging waste management of, in 1985,[519] but it was not able to bring about the effective harmonization of national policies. Only some EU Member States introduced measures on packaging and packaging waste management with a view to reducing environmental impact. As a consequence, diverging national legislation appeared in several Member States. For these and other reasons, Member States and economic operators approached the Commission to introduce comprehensive legislation on packaging.

In 1992, the Commission came forward with a proposal for a Council Directive on Packaging and Packaging Waste. Following a prolonged discussion in the European Parliament and the Council of Ministers, Directive 94/62/EC [520] was adopted.

This Directive's purpose is to harmonize national measures in order to prevent or reduce the impact of packaging waste on the environment. It contains provisions on the prevention, recovery, and recycling of packaging waste.

In 2004, the Directive was reviewed to provide criteria clarifying the definition of the term "packaging" and increase the targets for recovery and recycling of packaging waste. It established percentage targets for the recovery of packaging waste and the essential requirements that all packaging must meet.

In 2005, the Directive was revised again to allow new Member States transitional periods for attaining the recovery and recycling targets.

7.4 End of Life Vehicles Directive

The Directive 2000/53/EC[521] aims to prevent waste from vehicles and sets out measures for the reuse, recycling, and other forms of recovery of end-of life vehicles and their components. Preventive measures intend to reduce disposal of waste and improve the environmental performance of the economic operators involved in the life cycle of vehicles.

519. Council Directive 85/339/EEC of 27 June 1985 on containers of liquids for human consumption (Official Journal of the European Communities No L 176 of 6.7.85).
520. Directive 94/62/EC of European Parliament and Council of 20 December 1994 on packaging and packaging waste (Official Journal of the European Communities No L 365 of 31.12.1994). Amended by Directive 2004/12/EC, Directive 2005/20/EC, and Regulation (EC) No 219/2009.
521. Directive 2000/53/EC of the European Parliament and of the Council of 18 September 2000 on end-of life vehicles - Commission Statements (Official Journal of the European Communities No L 269 of 21.10.2000).

7.5 Waste Incineration Directive

The Waste Incineration Directive 2000/76/EC[522] aims to minimize environmental and human health impact on the of air, land and water emissions caused by the incineration or co-incineration of hazardous and non-hazardous waste. Co-incineration plants are installations whose main purpose is to produce energy or goods and which use waste as a regular or additional fuel source.

The Directive sets stringent operating conditions, technical requirements and emission limit values. All incineration or co-incineration plants must be permitted and the permit lists the categories and quantities of hazardous and non-hazardous waste that may be treated. Strict rules are imposed on the process to retain the waste at a sufficient temperature to guarantee complete waste combustion.

7.6 Other Directives of some importance are:

- Directive 2002/96/EC of 27 January 2003 regarding waste electrical and electronic equipment (WEEE);
- Directive 2006/21/EC of the European Parliament and of the Council of 15 March 2006 on the management of waste from extractive industries and amending Directive 2004/35/EC;
- Directive 2006/66/EC of the European Parliament and of the Council of 6 September 2006 on batteries and accumulators and waste batteries and accumulators and repealing Directive 91/157/EEC.

8. Italian rules on ABPs and on waste from ABPs

In Italy, the EU rulings on waste, particularly the Directive n. 2008/98/CE, were absorbed by the Legislative Decree n. 152/2006.[523] The Article 185, point 2b, excludes, from the scope of that decree, "the ABPs, including processed products (e.g. meat and bone meal), provided for by the Council Directive n. 1774/2002 (now Council Directive n. 1069/2009, in force from March 4, 2011, above mentioned), except those destined for incineration, for disposal in landfills or for use in a biogas or composting plant". The Article 184-bis excludes from the discipline of the same decree the "ABPs" when the following conditions occur:

- the by-product (substance or object) is originated from a production process whose primary purpose is not the production of the same;
- the by-product will certainly be used in the same or another production cycle;
- the by-product will be used directly without any further treatment;
- further use is lawful, with no impact on the environment and health.

The Article 184-ter lays down that "a waste ceases to be waste when it is subjected to a recovery operation, including recycling and preparing for re-use and meets the specific criteria to be adopted in accordance with the following conditions:

522. Directive 2000/76/EC of the European Parliament and of the Council of 4 December 2000 on the incineration of waste (Official Journal of the European Communities No L 332 , 28/12/2000).
523. Legislative Decree 3 April, 2006, n. 152, part IV, "Rules on waste management and reclamation of contaminated sites" (G. U. n. 88 del 14 April 2006, S. O. n. 96/L), then amended several times.

- substance or object is commonly used for specific purposes;
- a market or a demand exists for such a substance or object;
- substance or object fulfills the technical requirements for specific purposes and meets the existing legislation and standards that are applicable to products;
- the use of the substance or object will not have negative impacts on the environment or on human health".

However, the Regulation (EC) n. 1069/2009[524] contains several provisions that overlap or are in addition to those of the Legislative Decree n. 152/06. The Article 4 of Regulation lays down that "Member States shall ensure that an adequate system is in place on their territory ensuring that animal by-products are collected, identified and transported without undue delay and treated, used or disposed of in accordance with this Regulation". According to the articles 12, 13 and 14, Categories 1, 2 and 3 materials, will be directly disposed of as waste by incineration or co-incineration without prior processing or following processing, by pressure sterilization if the competent authority so requires, and permanent marking of the resulting material.

Category 1 material will be directly disposed of as waste by incineration without prior processing or following processing, by pressure sterilization if the competent authority so requires, and permanent marking of the resulting material. In the case of Category 1 material other than material referred to in article 8[525] will be disposed of by pressure sterilization processing, permanent marking of the resulting material and burial in an authorized landfill. Category 1 material referred to in article 8(f),[526] will be disposed of by burial in an authorized landfill or used as a fuel for combustion with or without prior processing.

Category 2 material will be disposed of in an authorized landfill, following processing by pressure sterilization and permanent marking of the resulting material or will be used for the manufacturing of organic fertilizers or soil improvers to be placed on the market in accordance with article 32 (marketable organic fertilizers and soil improvers) following processing by pressure sterilization, when applicable, and permanent marking of the resulting material or will be composted or transformed into biogas.

The Category 3 material will be used for the production of raw petfood, to be placed on the market in accordance with article 35 (other derived products placed on the pet food market); will be composted or transformed into biogas or will be used as a fuel for combus-

524. This Regulation, having the force of law in Italy, was increased by Legislative Decree 1 October, 2012 , n. 186, "Disciplina sanzionatoria per la violazione delle disposizioni di cui al regolamento (CE) n. 1069/2009 recante norme sanitarie relative ai sottoprodotti di origine animale e ai prodotti derivati non destinati al consumo umano e che abroga il regolamento (CE) n. 1774/2002, e per la violazione delle disposizioni del regolamento (UE) n. 142/2011 recante disposizioni di applicazione del regolamento (CE) n. 1069/2009 e della direttiva 97/78/CE per quanto riguarda taluni campioni e articoli non sottoposti a controlli veterinari in frontiera" (GU n. 255 del 31 ottobre 2012).

525. Animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed and animals killed in the context of TSE eradication measures.

526. Animals and parts of animals, other than those referred to in article 8 or article 10, that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes; foetuses; oocytes, embryos and semen which are not destined for breeding purposes; and dead-in-shell poultry.

tion with or without prior processing, or will be applied to land without processing in the case of raw milk, colostrum and products derived therefrom, which the competent authority does not consider to present a risk of any disease communicable through those products to humans or animals.

Any operator, establishment, or plant that generates, transports[527], handles, processes, stores, places on the market, distributes, uses, or disposes ABPs or derived products must be registered before commencing operations using a commercial document[528] or, when required by the EU ABPs Regulation or by a measure adopted in accordance with paragraph 6 by a health certificate. Commercial documents and health certificates accompanying ABPs or derived products, during transport have to include information on the origin, the destination, and the quantity of such products, and a description of the animal by-products or derived products and their marking, when such marking is required.

According to Article 21, operators will ensure that ABPs and derived products are accompanied during transport by a commercial document or, when required by this Regulation, or by a health certificate. With information on the origin, the destination and the quantity of such products, and a description of the ABPs or derived products and their marking, when such marking is required by this Regulation.

Operators will collect, transport and dispose of Category 3 catering waste in accordance with national measures foreseen in Article 13[529] of Directive 2008/98/EC (on waste).

According to Article 22, operators consigning, transporting or receiving animal by-products or derived products will keep a record of consignments and related commercial documents and health certificates. Operators will have systems and procedures in place to identify the other operators to which their ABPs or derived products have been supplied and the operators from whom they have been supplied.

This information will be made available to the competent authorities on request..

According to Article 23, with a view to registration, operators will before commencing operations, notify the competent authority of any establishments or plants under their control which are active at any stage of the generation, transport, handling, processing, storage, placing on the market, distribution, use or disposal of ABPs and derived products; provide the competent authority with information on the Category of ABPs or derived products un-

527. The use of bulk trailers which have a partition across the width of the camion, known as "split trailers", has an economic advantage to the industry by enabling the collection of different categories of by-product in a single vehicle movement. The Regulation 142/2011, Annex VIII Chapter II, states that all necessary measures must be taken to ensure that consignments of animal by-products and derived products are identifiable, kept separate and identifiable during collection where the animal by-products originate and during transportation.

528. The commercial document could be in accordance with the model set out under Commission Regulation (EU) No 255/2013 of 20 March 2013 amending, for the purposes of adaptation to scientific and technical progress, Annexes IC, VII and VIII to Regulation (EC) No 1013/2006 of the European Parliament and of the Council on shipments of waste (Official Journal of the European Communities L 79 of 21.3.2013) and shall accompany ABPs and derived products.

529. Member States shall take the necessary measures to ensure that waste management is carried out without endangering human health, without harming the environment and, in particular:
- without risk to water, air, soil, plants or animals;
- without causing a nuisance through noise or odours; and
- without adversely affecting the countryside or places of special interest.

der their control and the nature of the operations performed using ABPs or derived products as starting material.

The Chapter V - Collection, transport, identification and traceability – of the EU Commission Regulation 142/2011 lays down, in the article 17 (requirements regarding commercial documents and health certificates, identification, the collection and transport of ABPs and traceability), that the:

1. Operators will ensure that ABPs and derived products:
   a. comply with the requirements for collection, transport and identification set out in Chapters I (collection and transport)[530] and II (Identification)[531] of Annex VIII;
   b. are accompanied during transport by commercial documents and health certificates in accordance with the requirements set out in Chapter III (commercial documents and health certificates)[532] of Annex VIII (Amended by the Commis-

530. As from the starting point in the manufacturing chain referred to in Article 4 (1) of Regulation (EC) No 1069/2009, ABPs and derived products must be collected and transported in sealed new packaging or covered leak-proof containers or vehicles. Vehicles and reusable containers, and all reusable items of equipment or appliances that come into contact with ABPs or derived products, must be maintained in a clean condition. Reusable containers must be dedicated to the carriage of a particular animal by-product or derived product to the extent necessary to avoid cross-contamination. Packaging material must be disposed of, by incineration or by other means in accordance with Union legislation. Other provisions deal with the temperature conditions of the transport of ABPs, derogation for collection and transport of Category 3 material comprising of milk, milk-based products and milk-derived products, and of manure.

531. All necessary measures must be taken to ensure that: consignments of ABPs and derived products are identifiable and kept separate and identifiable during collection; consignments of ABPs and derived products are dispatched from one Member State to another Member State in packaging, containers or vehicles which are prominently and, at least for the period of transport, indelibly colour-coded for displaying information. The label attached to the packaging, container or vehicle must clearly indicate the category of the animal by-products or of the derived products and bear the following words visibly and legibly displayed on the packaging, a container or vehicle, as applicable:
- in the case of Category 3 material, "not for human consumption";
- in the case of Category 2 material and derived products from Category 2 material, "not for animal consumption";
- in the case of Category 1 material and derived products from Category 1 material where they are destined for disposal, "for disposal only";
- the manufacture of petfood, "for manufacture of pet food only";
- the manufacture of a derived product, "for manufacture of derived products only. Not for human or animal consumption or for application to land";
- in the case of gelatine produced from Category 3 material, "gelatine suitable for animal consumption";
- in the case of collagen produced from Category 3 material, "collagen suitable for animal consumption";
- in the case of raw petfood, "as pet food only";
- etc., etc.

532. During transportation, a commercial document or, when required by this Regulation, a health certificate must accompany ABPs and derived products. As already mentioned earlier, the commercial document could be in accordance with the model set out under Commission Regulation (EU) No 255/2013 of 20 March 2013 amending, for the purposes of adaptation to scientific and technical progress, Annexes IC, VII and VIII to Regulation (EC) No 1013/2006 of the European Parliament and of the Council on shipments of waste - Official Journal of the European Communities L 79/19 of 21.3.2013. It must be produced at least in triplicate (one original and two copies). The original must

sion Regulation (EU) No 255/2013 of 20 March 2013).

2. Operators consigning, transporting or receiving ABPs or derived products will keep records of consignments and related commercial documents or health certificates in accordance with the requirements set out in Chapter IV (Records)[533] of Annex VIII.

The Directive 2008/98/EC on waste, in turn, lays down that the establishments, the producers of hazardous waste and the establishments and undertakings which collect or transport hazardous waste on a professional basis, or act as dealers and brokers of hazardous waste, will keep a chronological record of the quantity, nature and origin of the waste, and, where relevant, the destination, frequency of collection, mode of transport and treatment method foreseen in respect of the waste, and will make that information available, on request, to the competent authorities. For hazardous waste, the records will be preserved for at least three years except in the case of establishments and undertakings transporting hazardous waste that must keep such records for at least 12 months. Documentary evidence that the management operations have been carried out will be supplied at the request of the competent authorities or of a previous holder (Article 35).

The Article 190 of the Italian Legislative Decree n. 152/06 lays down that the public and the private producers of hazardous and non-hazardous waste which carry out disposal or recovery of waste and/or produce waste from the same activity, the traders of waste, those who collect or transport special waste[534], if they do not adopt the "Waste Traceability System" (WTS),[535] must keep a stock book (record of loading and unloading).[536] The Article 193 lays down that the public bodies and the enterprises, which collect and transport waste, if they do not adopt the WTS, must use a "formulary of identification" (a kind of "commercial document") in which they must specify the name and address of the producer, the holder and the receiver of the waste, the origin, amount and type of waste, the date and the way forward for the transport. The formulary will be completed in four copies. Two copies are used by the carrier and accompany the waste.

The Article 212 of the same decree lays down that companies engaged in the collection and transportation of waste are obliged to be entered on the "National Registry of Environ-

accompany the consignment to its final destination. The receiver must retain it. The producer must retain one of the copies and the carrier the other. Member States may require that proof of the arrival of the consignments is provided by the TRACES system or by a fourth copy of the commercial document which is sent back by the receiver to the producer. The sender and carrier must keep copies for at least two years. Health certificates must be issued and signed by the competent authority.

533. The records shall contain a description of the animal species for Category 3 material and derived products therefrom, destined for use as feed material, the quantity of the material. In the case of records kept by any person consigning ABPs the records shall contain the date, the name and the address of the transporter and of the receiver. In the case of records kept by any person transporting ABPs the records must contain the date, the place of origin of the material, the name and the address of the receiver. In the case of records kept by any person receiving ABPs the records shall contain the date, the name and the address of the transporter and the place of origin of the material.

534. According to the Italian Legislative Decree n. 152/2006, Article 184, the "special waste" are those produced from agricultural activities, from industrial, craft, trade and services, from activities such as demolition, construction and excavations, from care activities.

535. In Italian "Sistema di tracciabilità dei rifiuti" (SISTRI), not yet operating in Italy.

536. Register similar to that adopted for recording the VAT.

mental Managers" (Albo Nazionale Gestori Ambientali).[537] It is not possible to carry out the collection and transportation of waste without that registration.

The various cases which arise in practice are also regulated in Italy by the "Guidelines[538] for the application of EU ABPs Regulation 1069/2009", but not everything is clear. What it is not clear?

The Article 6 (communication of vehicles and reusable containers) of the Guidelines, point 1, lays down that any undertaking which carries ABPs and derived products … must notify the Veterinary Service of the Local Health Authority (LHA)[539] in the area where the company is registered/recognized (headquarters). In addition, the list of vehicles and/or reusable containers placed under its control (model and license plate) and its variations must also notify the Veterinary Service of the Local Health Authority. The second point refers to the vehicles and/or reusable containers for the transport of ABPs and derived products. The previous point, however, states that WHAT can not be used for the transport of live animals, food, feed and waste.

The point 1 of the Article 7 (Identification mode of vehicles and containers) lays down that the Veterinary Service of the LHA, upon receiving the notification referred to in Article 6, records in a register any vehicle or vessel used for the transportation of ABPs or derived products so as to provide an identification code.

According to the point 1 of the Article 8 (commercial document), the transport of ABPs and derived products must be accompanied by the Commercial Document referred to in Annex VIII, Chapter III of the EU Commission Regulation 142/2011. In order to establish local needs within their territory, egions may authorize for the Category 1, 2 and 3 materials: the use of a simplified Commercial Document. The choice of that option must be communicated to the Ministry of Health.

In the case of point 2,when ABPs and derived products are intended to be disposed of as waste, the Commercial Document must be replaced by the documentation required by environmental legislation, as in Article 10.

The point 1 of Article 9 (traceability/register) states that the persons consigning, transporting or receiving ABPs and/or derived products must keep record of consignments of Article 22 of EU ABPs Regulation 1069/2009 and related Commercial Documents or health certificates. In the subsequent points indicate the cases that do not require the maintenance of the register.

The point 1 of the Article 10 (method of disposal as waste - in accordance with environmental – of Category 1, 2 and 3 materials) lays down that disposal as waste of ABPs and derived products of Category 1, 2 and 3 materials must be made in the manner prescribed by environmental legislation as regards means of transport, waste formulary, and registration of waste or WTS in the following cases:

537. According to the Decree of the Ministry of the Environment 28 April 1998, n. 406 (Official Gazette of the Italian Republic no. 276 of 25 November 1998).

538. Agreement between the Italian Government, the Regions and the Autonomous Provinces of Trento and Bolzano on the document containing Guidelines for the application of the Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002.

539. In Italy called ASL (Azienda Sanitara Locale).

1.1. in incineration or co-incineration plants approved in accordance with environmental legislation (Article 6, point 1, letter a) of the Commission Regulation (EU) 142/2011);

1.2. in an authorized landfill in accordance with environmental legislations, following processing by pressure sterilization and permanent marking of the resulting material, if they are Category 1 materials, other than those referred to in Article 8, letter a), points i) and ii) and Category 2 materials.

1.3., 1.4., 1.5., 1.6., 1.7, in authorized landfills, the other points according to Article 10 of EU ABPs Regulation 1069/2009.

The point 2 of the same article also provides: "the ABPs or derived products of any Category, including the dead animal carcasses, during any subsequent phases of collecting from the place of production (storing, processing in facilities approved in accordance with EU ABPs Regulation 1069/2009) are still considered ABPs and therefore are subject to the requirements of the Regulations, including transport".

According to the Guidelines (point 1, Article 1 – Registration plants) "all activities of production, transport, handling, processing, storage, marketing, distribution, use and disposal of ABPs and derived products are subject to registration procedure, if authorization is not required, in accordance with Article 24 (Approval of establishments or plants)[540] of the EU ABPs Regulation 1069/2009 or, in the case of plants that generate by-products, if they have not already been approved or registered in accordance with Regulation No 853/2004."[541] Such registration (point 3) does not exempt the operator from the notification, if the ABPs or the derived products represent feed materials.

The operator (point 4) makes the notification of the opening, change in ownership or type of activity, cessation, closing of any activity subject to registration, at the LHA or the Single Desk for Productive Activities (according to the procedure established by each Region) where the business is located or in which he is resident (in the case the activity is devoid of establishment, such as the activity of transport for third parties or brokerage, etc.).

The registration is made after the notification (in a manner determined by individual Regions)[542]. The operator must declare that the activity meets the minimum requirements established the EU ABPs Regulation 1069/2009 and the Commission Regulation (EU) 142/2011. The notification will be accompanied by a Technical report and a Planimetry of premises. Registration for the transport activity (Article 23 del ABPs Regulation

540. Operators shall ensure that establishments or plants under their control are approved by the competent authority, where such establishments or plants carry out one or more of the following activities: processing of ABPs by pressure sterilization; disposal, as waste, by incineration of ABPs and derived products; disposal or recovery of ABPs and derived products, if they are waste, by co-incineration; use of ABPs and derived products as fuel for combustion; manufacturing of pet food; manufacturing of organic fertilizers and soil improvers; transformation of ABPs and/or derived products into biogas or compost; handling of ABPs after their collection, by way of operations such as sorting, cutting, chilling, freezing, salting, removal of hides and skins or of specified risk material; storage of ABPs.

541. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs (Official Journal of the European Communities L 139 of 30.4.2004).

542. Each registered plant or operator is placed on a national list of the Ministry of Health edited by the respective Region or LHA.

1069/2009) only applies to companies whose business is to transport of ABPs and derived products.

According to Article 2 (approval of establishments), the establishments or plants, where activities are carried out according to Article 24 of the EU ABPs Regulation 1069/2009, are subject to approval. The procedure for approval must comply with the provisions of Article 44 (Procedure for approval) of the EU ABPs Regulation 1069/2009. Operators, who wish with the use of implants, to engage in the activities, provided for in Article 44 of the EU ABPs Regulation 1069/2009, must submit an application for authorization and accordance with procedures established by the Region. The authorization document must specify:
activity carried out;

- type of product generated;
- category (Articles 8, 9 and 10 of EU ABPs Regulation 1069/2009);
- identification number.

The following types of plants are excluded from authorization and registration if they are subject to other national disposition:

- incineration or co-incineration (Italian Decree Lgs, May 11, 2005 n. 133 and Directive 2000/76/CE;
- approved landfills (Directive 1999/31/CE;
- establishments or plants that generate by-products whose activities have been approved or registered according to EU laws on food hygiene;
- biogas or compost plants outbuildings on to the farm when introducing manure;
- biogas or compost plants outbuildings on to the farm when introducing waste from kitchens and catering;
- biogas or compost plants outbuildings on to the dairy farms when they introduce ABPs from the treatment and processing of milk.

8.1 Conclusions

Summarization of the the situation follows. The management of ABPs is not subject to Italian Legislative Decree No 152/2006, except in cases when ABPs are destined for incineration, for disposal in landfills, or for use in a biogas or composting plant. Some questions arise immediately: when do the ABPs become waste? At the beginning of transport to the ultimate fate site for disposal/recovery? Or upon arrival at the plant? Moreover, if there is a stop at an intermediate site (also a treatment site), how should ABPs be stored and managed?

ABPs are normally produced at the slaughter centers. Some parts may be intended to go to a recovery center (mentioned above) others slated for disposal.

If all ABPs are destined to go to a selection and recovery center, and are in the presence of by-productsthat are excluded from the discipline of Italian Legislative Decree No 152/2006, the transport company would not have the obligation to enrollment in the National Registry of Environmental Managers. According to the EU ABPs Regulation 1069/2009, the Commission Regulation (EU) 142/2011 and the Guidelines, the company must utilize a commercial document during transport. The vehicles used must be marked with the identification code received from the LHA responsible for the area.

On the other hand, if they are considered waste and therefore destined for incineration, for disposal in landfills, or for use in a biogas or composting plant, the carrier is required to register with the National Registry of Environmental Managers (category 4 or 5, with the indication of the codes CER of the materials to be transported), use the "identification formulary" , and (until the WTS will be adopted) keep the "loading and unloading registry". Vehicles must be carriage authorized by the Regional Section of the Register cited. Dangerous ABPs (e.g. in the case of animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE or other type of dangerous diseases has been officially confirmed) must be listed in Category 5 and also possess a health certificate. According to the Guidelines (point 2 of Article 8), it is not necessary to have the commercial document expected by the EU ABPs Regulation 1069/2009 and Commission Regulation (EU) 142/2011 and their successive amendments. This view, however, seems inconsistent with the provisions of point 2 of Article 10 of the same Guidelines that, as already mentioned, lays down that "the ABPs or derived products of any category, including the carcasses of dead animals, in eventual successive stages to the collection from the production site (storing, processing in facilities approved by EU ABPs Regulation 1069/2009), are still considered ABPs and, therefore, subject to the obligations under the Regulation, transport included." If we follow these rules the transport operator would not have the obligation of registration in the National Registry of Environmental Managers, despite the point 1 of the Article 10 of the Guidelines.

The centers of selection and recovery (they can also be located near or in slaughter plants) as already mentioned must be in compliance with the provisions of the EU ABPs Regulation 1069/2009, the Commission Regulation (EU) 142/2011 and the Italian Guidelines. In particular they will hold the registration issued by LHA as a result of notification by the operator.

Operators carrying out both activities for which recognition is provided for in Article 24 of the EU ABPs Regulation 1069/2009 (both those provided by the Italian Legislative Decree n. 152/08), must ensure a permanent and absolute separation of the selection and recovery. As regards to transport, the same vehicle cannot carry both ABPs, as waste destined for disposal and ABPs or derived products intended for recovery and recycling. In other words, it does not seem possible to request registration and its identification code from the LHA and at the same time to ask the Italian Regional Section of the Registry of Environmental Managers, which has territorial jurisdiction, for the registration and the subsequent authorization of the same vehicle.

The Italian Court of Cassation, III Section penal, has intervened on the subject with several judgments (e.g. n. 2710 of 15 December 2011 and n. 25364 of 27 June 2012) ruling that the ABPs are not subject to the regulations regarding waste and are exclusively subject to EC Regulation n. 1774/2002, only if they are actually classified as by-products, according to the article 184 bis of Italian Legislative Decree n. 152/2006. Otherwise, if the producers discard the ABPs to send their to the disposal, they remain subject to the rules laid down by the latter decree.

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4.4 – Brain and spinal cord

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PART II – Treatment and legislation on ABPs and waste

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